Joint cartilage repair with transplantation of embryonic chondrocytes embedded in collagen-fibrin matrices

C. Perka, O. Schultz¹, K. Lindenhayn, R.-S. Spitzer, M. Muschik, M. Sittinger¹, G.R. Burmester¹

Abstract

Objective
The objective of this study was to assess the feasibility of transplanting embryonic chondrogenic cells within a collagen-fibrin substrate for the reconstitution of full-thickness cartilage defects in chicken knee joints.

Methods
Full-thickness cartilage defects were created mechanically on the weight-bearing surface of the tibial condyle in 45 adult chickens and subsequently filled with chondrocytes embedded in a chondrocyte-collagen-fibrin gel. The transplants were compared to untreated defects and collagen-fibrin transplants without cells. The results were analyzed using histochemical and morphometrical methods after 3, 12 and 24 weeks. A semiquantitative histological grading system was applied to evaluate the transplant integration and the newly formed cartilage architecture.

Results
Chondrocyte-gel grafts developed to hyaline-like cartilage without any granulation tissue in the interface after 3 weeks. After 12 weeks the defects in the experimental group were filled completely with hyaline cartilage. The defects in the control groups in all cases healed with fibrous repair tissue.

Conclusion
Fibrin-collagen gel allowed stable graft fixation and provided an adequate microenvironment for embryonic chondrocytes to generate hyaline-like neocartilage in a full-thickness cartilage defect.

Key words
Chondrocyte transplantation, cartilage repair, cell transplantation methods.

**Chondrocytes in collagen-fibrin-matrices / C. Perka et al.**

**Carsten Perka, MD; Olaf Schultz, MD; Klaus Lindenhayn, MD; Ron-Sascha Spitzer, MD; Michael Muschik, MD; Michael Suttinger, MD; Gerd R. Burmester, Prof. Dr. med., Director of Dept. of Rheumatology and Clinical Immunology, Humboldt University.**

This study was supported by a grant from the DFG (Deutsche Forschungsgemeinschaft) BU 445-5/2.

Preliminary results of this study have been previously published in Zeitschrift für Orthopädie und ihre Grenzgebiete [in German].

Please address correspondence and reprint requests to: Dr. med. Olaf Schultz, Schumannstraße 20/21, 10098 Berlin, Medizinische Klinik III der Charité, requests to: Dr. med. Olaf Schultz, Medizinische Klinik III der Charité, Schumannstraße 2021/1, 10998 Berlin, Germany. E-mail: olaf.schultz@charite.de

Received on March 10, 1999; accepted in revised form on July 6, 1999.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2000.

**Introduction**

Articular hyaline cartilage, although active in synthesis and degradation in response to a changing mechanical or biochemical environment, has limited capacity for repair in the adult. Thus, articular cartilage defects do not heal adequately and often progress to osteoarthritis (1-5). Various mesenchymal cells and tissues possess a chondrogenic potential depending on the local nutritional and mechanical environment. Osteochondral and osteoperiosteal grafts (6-8), periosteum (6, 9-11), perichondrium (12-14), isolated adult chondrocytes (15-17), embryonic chondrocytes (18, 19, 20) and mesenchymal cells (21) have all been investigated for their regenerative capacity in cartilage repair.

Alternative approaches for cartilage repair involve the transplantation of chondrogenic cells in bioresorbable polymer scaffolds (24-28) or hydrogel-substrates such as collagen (29-33) and fibrin (18, 34). These biodegradable carrier substrates provide temporary mechanical support, can deliver a large number of cells and allow the preformation of a cartilaginous implant of a defined shape in vitro. The criteria for a successful approach include: the easy and stable fixation of the implant in the defect, the maintenance of viability of the cells transplanted, and the synthesis of an appropriate matrix reproducing the mechanical and functional long-term properties (35).

Fibrin tissue adhesives have been widely used in orthopedic surgery (36, 37). Itay applied a mixture of fibrin and extracellular matrix produced in chondrocyte culture as a graft material (18). Noguchi and Wakitani used a collagen gel as a carrier for the transplantation of iso- and allogeneic chondrocytes, and they reported success rates of 75-100% during a 52-week follow-up period (21, 34, 38). A combination of collagen with fibrin matrix appeared to us to be a feasible solution addressing the problems of transplant fixation, the provision of a permissive microenvironment, and the long-term viability of the transplanted cells. In an initial model we utilized allogeneic embryonic chick chondrocytes for transplantation, since these cells possess a high proliferative and functional potential. The objective of our study was to assess the effects of collagen-fibrin-matrix in graft fixation and following calcification under the influence of proliferating embryonic cells in this specific microenvironment.

**Materials and methods**

**Experimental animals**

Forty-five 9 month old chickens were included in this study and divided into 3 groups: an experimental group and two control groups after randomization. Both tibiotarsal joints were dissected, and the untreated contralateral joint served for comparison.

**Isolation and preservation of embryonic chondrocytes**

The proximal and distal femoral and tibial epiphyses of 75 ten day old chicken embryos were dissected under sterile conditions and collected in cooled PBS. The tissue samples were incubated for 20 minutes at 37°C in a PBS-solution containing 1% trypsin and 0.2% EDTA (pH 7.7). They were then crushed in a homogeniser and again incubated in the PBS-trypsin solution for 20 minutes. The harvested cells were suspended in Ham’s F-12-medium supplemented with 10% fetal calf serum (FCS), passed through a 20 µm polyester filter (Estal mono, Seidengazenfabrik AG Thal, Switzerland) and washed three times with Ham’s F-12 medium.

6.6 x 10^7 cells were transferred to 135 mm² dishes coated with 0.5% agarose and cultured in Ham’s F-12-medium with 10% FCS and 0.3% tryptose phosphate broth. All of the cell culture media contained 100 units/ml penicillin, 100 µg/ml streptomycin and 0.5 µg/ml fungizone.

Following a culture period of 10 days at 37°C and 5% CO₂, the cells were harvested after disintegration of the cell aggregates with 0.1% collagenase in PBS for 60 minutes at 37°C. The trypan blue dye exclusion test proved that more than 90% of the cells were viable. After repeated washing procedures the cells were embedded in a collagen fibrin gel.

**Preparation of the matrix**

The frozen skins of 5 young chickens were cut into small pieces. A water- and
neutral salt-extraction step, as well as an acid extraction with 0.5 M acetic acid, were performed and the lipids were removed by passage through a Celite filter (type 545). All of the extraction steps were performed at 5°C in the presence of 100 µg/ml streptomycin and 100 units/ml penicillin. After precipitation, 2.8 g of purified sterile collagen was obtained.

Preparation of fibrinogen

750 mg fibrinogen (Sigma F-4753) was dissolved in 50 ml of distilled water, sterile filtered and freeze dried. The component A of the glue contained 50 mg fibrinogen per ml DMEM as well as 1000 KIU Trasylol (Aprotinin, Bayer AG, Leverkusen, Germany); component B contained 200 - 300 IU thrombin/ml DMEM (Behringwerke, Frankfurt/Main, Germany).

Embedding of chondrocytes in the collagen-fibrin-gel

A mixture of 0.4 ml 9 x DMEM, 2.0 ml 0.4% collagen solution in 0.005 M acetic acid and 1.2 ml of a fibrinogen solution (322 mg in 2.5 ml water with 0.1 ml Trasylol (2000 KIU) was prepared on ice. The cells (7.5 x 10⁶ chondrocytes/ml) were suspended in 3 ml of this solution, and finally 0.3 ml of a thrombin solution was added (1,000 - 1,200 IU thrombin; 4 ml 164 µM CaCl₂-solution; 4 ml 2 x DMEM). The suspension was transferred immediately into a multi-well plate (diameter 16 mm, gel thickness 5 mm). The final cell density in the gel was 2.27 x 10⁶ cells/ml.

Surgical procedure

A total of 45 chickens were operated on. No animal had to be excluded from the study because of death, infection or technical failure. Under general anesthesia and sterile conditions the left tibiotarsal joints were exposed through a medial arthrotomy incision, to provide exposure of the lateral and medial joint compartment. A full thickness defect in the articular cartilage and subchondral bone (1.7 mm in diameter) with an average depth of 2.5 mm (1.3 - 3.1 mm) was made with a manual drill in the weight-bearing area of the medial condyle. The weight-bearing area of the tibia was defined as the area which was in contact with the talus in the neutral position. This was used as a standardized option for defect simulation in a permanently mechanically stressed zone. In the experimental group the defects were filled with component B and the chondrocyte-collagen-fibrin cylinder was immersed in component A. Subsequently this cylinder was pressed to the defect. In the first control group the defects were filled with collagen-fibrin gel without chondrocytes, while in the second group the defects were left empty. The contralateral tibiotarsal joint served as a control for histological comparison. All animals were kept in metal cages after the operation, at a constant room temperature of 22°C and natural light cycle. Five chickens from each group were sacrificed at 3, 12 and 24 weeks post-operatively.

Histological and histochemical evaluation

The tibiotarsal joints were dissected after complete removal of the soft tissues. After the macroscopic evaluation, the tibial condyles were fixed immediately in a 4% formaldehyde-PBS solution (pH 7.2) containing 0.5% cetylpiridinium chloride. After decalcification with formic acid, the specimens were dehydrated and subsequently embedded in paraffin. 5 µm thick sagittal sections through the distal tibia were stained with hematoxylin-eosin (HE), Masson-Goldners and Alcian blue staining (pH 1.0) for sulphated glycosaminoglycans.

Healing indices

All sections from each animal were examined and scored independently by 3 persons who were blinded to the type of operation and the time lapse after the operation, of the samples. All samples were evaluated using a histological scale based on Pineda et al. (39) and Wakitani (21) (Table I). The morphology of the implant was graded into 5 categories.

Histomorphometrical evaluation

The cell density and mitotic rate were evaluated histomorphometrically. The region corresponding to the intermediate zone was chosen for cell counting, since it is clearly defined in all the developmental stages of the implant. The statistical analysis included the t-test and the Wilcoxon’s rank sum test performed by the computer-assisted software program STATPAL®.

Results

Macroscopic observations

All defects were induced in the full weight-bearing area in order to compare a similar biomechanical situation in all animals. The arthrotomy wound healed completely without complications in all animals within one week. On gross examination the joints in the experimental group revealed no signs of osteoarthritis, inflammatory reaction, proliferation of synovium, or cartilage destruction. The defects were completely filled with smooth, white, glistening tissue reaching the level of the surrounding cartilage. In all cases the original defect was clearly marked after 3 weeks. At 12 weeks the defect had become opaque, the articular

<table>
<thead>
<tr>
<th>Category</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td></td>
</tr>
<tr>
<td>Hyaline cartilage</td>
<td>0</td>
</tr>
<tr>
<td>Mostly hyaline cartilage</td>
<td>1</td>
</tr>
<tr>
<td>Mostly fibrocartilage</td>
<td>2</td>
</tr>
<tr>
<td>Mostly non cartilage</td>
<td>3</td>
</tr>
<tr>
<td>Non-cartilage only</td>
<td>4</td>
</tr>
<tr>
<td>Matrix-staining (metachromasia)</td>
<td></td>
</tr>
<tr>
<td>Normal (compared with adjacent host cartilage)</td>
<td>0</td>
</tr>
<tr>
<td>Slightly reduced</td>
<td>1</td>
</tr>
<tr>
<td>Markedly reduced</td>
<td>2</td>
</tr>
<tr>
<td>No metachromatic stain</td>
<td>3</td>
</tr>
<tr>
<td>Surface regularity</td>
<td></td>
</tr>
<tr>
<td>Smooth (&gt; 3/4)</td>
<td>0</td>
</tr>
<tr>
<td>Moderate (1/2 - 3/4)</td>
<td>1</td>
</tr>
<tr>
<td>Irregular (1/4 - 1/2)</td>
<td>2</td>
</tr>
<tr>
<td>Severely irregular (&lt; 1/4)</td>
<td>3</td>
</tr>
<tr>
<td>Thickness of cartilage</td>
<td></td>
</tr>
<tr>
<td>&gt; 2/3</td>
<td>0</td>
</tr>
<tr>
<td>1/3 - 2/3</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 1/3</td>
<td>2</td>
</tr>
<tr>
<td>Integration of donor with adjacent host cartilage</td>
<td></td>
</tr>
<tr>
<td>Both edges integrated</td>
<td>0</td>
</tr>
<tr>
<td>One edge integrated</td>
<td>1</td>
</tr>
<tr>
<td>Neither edge integrated</td>
<td>2</td>
</tr>
<tr>
<td>Total maximum</td>
<td>14</td>
</tr>
</tbody>
</table>

Table I. Histological grading scale for the defects of cartilage (modified after Pineda and Wakiitani, refs. 38, 32).
cartilage was smooth and white, and the margins of the transplant were hardly distinguishable. After 24 weeks the implant appeared to integrate well with the surrounding normal cartilage.

In contrast, the animals from the control group showed signs of cartilage erosion around the defects associated with mild synovitis. The defects were characterized by incomplete or no healing at all times; no differences between the two control groups were observed. The surrounding cartilage was of a dull, pale yellow color. The defects were either empty or filled partially with fibrous tissue and the surfaces appeared irregular. At 24 weeks the defect area had expanded due to degeneration of the adjacent cartilage.

**Histological observations**

**Normal cartilage.** Itay et al. have provided a histologic description of normal rooster articular cartilage (18). The cartilage layer of tibial condyle is 0.35 ± 0.1 mm thick and consists of three zones: the superficial zone, the intermediate zone and the deep zone (Fig. 1 a-b).

**Experimental group.** Three weeks after surgery, the defects containing the embryonic chondrocyte implants were filled with hyaline cartilage-like tissue. A high density of small round cells with high mitotic activity was observed, accompanied by an increase in the cell number and a relative decrease in the intercellular matrix (Fig. 2a). No necrosis was observed in the entire transplant area. The newly formed matrix stained positively on Masson-Goldner staining (Fig. 2b). No inflammatory reaction or granulation tissue was observed at the clearly demarcated boundary between the original cartilage and the transplant (Fig. 2c). The intercellular matrix stained intensely with Alcian blue, with a marked staining of the intermediate and deep zones, whereas the matrix production in the superficial zone was less pronounced (Fig. 2d). In the deeper zone the chondrocytes tended to cluster and undergo hypertrophy. No newly formed cancellous bone was observed in this area.

Twelve weeks after surgery, the newly formed cartilage completely filled the defects. The number of cells and the mitotic rate were decreased in all zones. The outermost surface consisted of a thin layer with a fibrillar pattern. Nearly complete repair and integration of the implant was observed. The implant could be divided into two areas (Fig. 3a). In the superficial zone the transplant had the appearance of hyaline articular cartilage, with an identical morphological pattern. A cartilage to bone transformation was observed in the deep area. Primary osteons had developed from hypertrophic chondrocytes and were visible around the invading vascular elements (Fig. 3b). At the interface of the implant to the original cartilage and bone no granulation tissue was observed. Numerous vascular elements surrounded the implant in the deep zone. The entire matrix of the superficial zone was intensely stained with Alcian blue. The staining was less prominent compared to 3 weeks after the implantation, but was comparable to normal cartilage, whereas the deeper zone revealed weaker staining. Distinguishing features of the implant in comparison to the original cartilage were a higher cell density, more intense Alcian blue matrix staining, a lower level of collagen expression, and a different structure of the subchondral bone (Fig. 3c).

Twenty-four weeks after surgery, the implant was completely integrated. Hyaline cartilage tissue was observed in the intermediate and upper zones, whereas in the superficial zone the progressive development of fibrocartilage accompanied by a decrease in the cell number was notable (Fig. 4 a-b). The overall thickness of the hyaline cartilage was reduced compared with the defects after 12 weeks. Alcian blue staining revealed no difference between the original cartilage and the transplant. In the deeper zone the cartilage was completely transformed to bone. Of note, the histological appearance was comparable to normal hyaline cartilage.
Untreated defect group. In the control group with the untreated defects, progressive filling with soft tissue was observed in all cases. After 3 weeks vascularization at the margins of the defects had initiated and collagen fibers were observable in the intercellular spaces. The defects contained abundant spindle-shaped fibroblasts and were surrounded by pathologically changed cartilage tissue (Fig. 5).

Collagen-fibrin defect group. In this group the original filling material was partially resorbed. No cells could be observed within the remnants of the gel. Connective tissue with scattered fibroblasts filled the resorption areas. There were no significant differences between the untreated and collagen-fibrin filled defects after 12 and 24 weeks. No defect healed with hyaline cartilage. All defects were filled with fibrous scar tissue containing only small amounts of metaplastic cartilage, abundant fusiform fibroblasts and small blood vessels at the margins (Fig. 6 a-b). No metachromatic Alcian blue matrix staining was observed (Fig. 6c). In general, the defects consisted of reconstructed subchondral bone in the deeper zone and fibrous cartilage in the superficial area.

Histological grading scale
A grading scale was used for the histological evaluation of the repair tissue. The scores for the experimental group were improved at 12 weeks compared to those at 3 weeks. However, after 24 weeks the scores decreased because of cartilage except for the progressively irregular surface.

Fig. 2. (a) Hyaline-like cartilage 3 weeks after implantation. The defect is completely filled by the implant (HE, original magnification x20).
(b) Collagen fibers 3 weeks after implantation. The extracellular matrix contains only a few collagen fibrils (arrow) (Masson-Goldner, original magnification x200).
(c) The interface between the newly formed and the original cartilage is clearly marked (arrow). No granulation tissue can be seen. (Masson-Goldner, original magnification x100).
(d) Synthesis of proteoglycans 3 weeks after transplantation. An intensely stained intercellular matrix can be seen in the intermediate and deeper zones (Alcian blue, original magnification x100).
Fig. 3. (a) Histological evaluation 12 weeks after the implantation shows well differentiated cartilage completely filling the defect. Cartilage to bone transformation is visible in the deeper zones (arrow), whereas the maturation of hyaline cartilage is observable in the superficial area (Masson-Goldner, original magnification x50). (b) Cartilage maturation with the distribution pattern of collagen fibrils in the superficial zone 12 weeks after transplantation. Cartilage to bone transformation with invading vascular elements, hypertrophic chondrocytes and developing primary osteons in the deeper zone can be seen (Masson-Goldner, original magnification x100). (c) The chondrocytic transplant after 12 weeks revealed matrix staining comparable to normal cartilage. In the deeper zones weaker staining was observed (Alcian blue, original magnification x20).

Fig. 4. (a) Cartilage maturation 24 weeks after implantation. A thick layer of collagen fibers can be seen on the surface (arrow) (Masson-Goldner, original magnification x30). (b) Status of implant transformation 24 weeks after implantation. An increased loss of chondrocytes in the superficial zone can be observed (HE, original magnification x100).
surface irregularity and cell morphology changes. At each time period, the histological scores of the control groups were distinctly lower compared to the experimental group. The results of the histological grading corresponded very well with the microscopic and macroscopic observations. Thus, the histological scores for the control groups reflected inferior cartilage repair in comparison to the experimental group (Table II).

**Morphometrical results**

The cell density and the mitotic rate were calculated by counting the number of nuclei per standard square and the number of mitoses visible in this square. The mean value for the mitotic rate decreased significantly from 7.9% (3 weeks after surgery) to 2.8% (at 12 weeks) and 0.9% (24 weeks). The cell density at various times after transplantation is shown in Figure 7.

**Discussion**

Strategies involving cell transplantation for tissue repair face various problems: (1) the use of cells capable of restoring tissue structure and function; (2) the creation of a conductive microenvironment providing optimal conditions for graft survival and integration; and (3) the development of modalities for a simple

---

**Fig. 5.** Granulation tissue formation 3 weeks after implantation in an untreated defect. The defect is only partially filled. In the deeper area invading soft connective tissue is visible (Masson-Goldner, original magnification x20).

**Fig. 6.** (a) Dense fibrous tissue 24 weeks after surgery in a collagen-fibrin treated defect (Masson-Goldner, original magnification x20). (b) Collagen-fibrin treated defect after 24 weeks. In the superficial zone the parallel orientation of spindle shaped fibroblasts may be seen (arrow). (Masson-Goldner, original magnification x200). (c) 24 weeks after the operation fibrous scar tissue can be seen filling the collagen-fibrin treated defect (Alcian blue, original magnification x30).
Chondrocytes in collagen-fibrin-matrices / C. Perka et al.

Table II. Results of the histological grading score. Following Wakitani (34) statistical analysis was not performed because the grading system is not linear or comparably equivalent between the five categories.

<table>
<thead>
<tr>
<th>Defect filling</th>
<th>Time after implantation (wks.)</th>
<th>Cell morphology</th>
<th>Matrix staining</th>
<th>Surface regularity</th>
<th>Thickness of cartilage</th>
<th>Integration of transplant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>3</td>
<td>0.8</td>
<td>0.4</td>
<td>1.6</td>
<td>1.2</td>
<td>0.8</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.8</td>
<td>0.4</td>
<td>1.0</td>
<td>0.4</td>
<td>0.6</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.2</td>
<td>0.8</td>
<td>1.8</td>
<td>1.4</td>
<td>0.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Control group (empty defects)</td>
<td>3</td>
<td>3.6</td>
<td>2.6</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.4</td>
<td>2.2</td>
<td>2.6</td>
<td>1.6</td>
<td>1.6</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2.0</td>
<td>2.0</td>
<td>2.2</td>
<td>1.2</td>
<td>1.0</td>
<td>8.4</td>
</tr>
<tr>
<td>Control group (collagen-fibrin-gel alone)</td>
<td>3</td>
<td>3.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.0</td>
<td>2.0</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.2</td>
<td>2.6</td>
<td>2.0</td>
<td>1.4</td>
<td>1.8</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2.0</td>
<td>2.2</td>
<td>2.2</td>
<td>1.4</td>
<td>1.2</td>
<td>9.0</td>
</tr>
</tbody>
</table>

and flexible surgical reconstructive approach.

In this study, the process of chondrogenesis (the remodeling of cartilage and subchondral bone) driven by transplanted embryonic chondrocytes in a collagen-fibrin matrix was investigated over a 24-week period in a chicken defect model. Embryonic chondrocytes were chosen for cartilage transplantation because of their superior proliferative activity and their differentiation potential (18-20) due to the increased synthesis of morphogenic factors including FGFs, TGF-β and BMPs, and an enhanced expression of the corresponding receptors (40-42). It may be speculated that these cells are more sensitive to signals from the surrounding microenvironment promoting tissue regeneration.

In this animal model, the histomorphological changes following chondrocyte transplantation and further maturation were mainly influenced by the specific microenvironment, as demonstrated by the zonal and sequential remodeling of the defect area. The implanted cells in the superficial zone nourished by the synovial fluid developed to hyaline cartilage, whereas the chondrocytes in the deeper regions of the implant in contact with blood transudation underwent hypertrophic transformation to bone tissue 12 weeks after transplantation. The superficial hyaline-like cartilage consisted of a high number of well-differentiated chondrocytes, thus documenting the excellent viability and functional capacity of the implanted cells. The initially increased chondrocyte proliferation, possibly due to the early stage of development of the embryonic cells, decreased to normal values during the observation period as a sign of maturation. Thus, the evolution of the transplant illustrated the stages already described by Itay et al.: proliferation, maturation and transformation (18).

In our view it was quite significant and appears to be the only explanation that the observed repair with hyaline-like cartilage was due to the transplantation of embryonic chondrocytes within their carrier substrate. In comparison, neither the matrix substrate alone nor untreated defects in the control groups showed signs of a sufficient defect repair indicative of alternative regeneration pathways.

Chondrocyte amplification prior to transplantation was performed in a three-dimensional cell culture in agarose gel to prevent fibroblastic-like differentiation and the loss of specific matrix production resulting from prolonged monolayer culture (43, 44). For the subsequent chondrocyte transplantation, a collagen-fibrin matrix was applied which proved to serve as a suitable vehicle to support neocartilage formation and is in line with other experimental attempts (33, 34). Both substrates are biodegradable, not cytotoxic and favor cell proliferation and allow chondrocytic differentiation. Collagen substrates for chondrocyte transplants, as used by Wakitani et al. (32, 37), demonstrated successful healing of full thickness defects in rat and rabbit knees. In addition, we used a combination which included a fibrin substrate, due to its well-known properties in reconstructive surgery supporting graft adhesion and fixation (18, 34-36, 45-50). Thus, we observed a complete bonding between the transplant and adjacent cartilage without any inflammatory reac-
tion, which is in contrast to other studies (51).

Britberg et al. have developed and clinically tested a method to transfer chondrocytes into human cartilage defects (22, 23). Their procedure includes the initial surfacing of the cartilage defect with a periosteal tissue flap fixed with sutures in the surrounding cartilage tissue. Subsequently, a suspension containing the amplified and cultured chondrocytes is injected into the defect. The homogeneous distribution of cells in the defect area is achieved by dissolution in a liquid matrix substance. Although the procedure used in our study was also based on the transplantation of chondrocytes homogeneously distributed in a semi-liquid substrate, technical modifications made it more efficient. Thus, matrix polymerization with the application of the fibrin-thrombin system directly during transplantation made the additional periosteal flap unnecessary. As a consequence optimal fixation and excellent contact at the edges (border zone) to the surrounding tissue was established. This technique is clearly advantageous because of the rationalisation of the surgical procedure and the prevention of additional trauma by suture fixation of the periosteal flap (fixation of the transplant requires an extended arthroscopy and may lead to mechanical damage of adjacent cartilage). Furthermore, our approach circumvents a possible disintegration of the periosteal flap. The initial fibrocartilaginous transition of the superficial layer after 24 weeks could indicate normal structural changes rather than a degenerative transformation. This may be due to the different architecture of the avian joint cartilage. A rejection of the transplanted allogeneic embryonic cells at the cartilage surface could have an additional impact on these changes. Nevertheless, the transplantation of epiphyseal embryonic chondrocytes significantly increased the amount of newly formed cartilage tissue compared to the control groups.

Our study was limited to 24 weeks because cells switched from the proliferative stage to chondrogenic differentiation within this period. However, we do consider that it will be necessary to extend this period for the evaluation of the biocompatibility, mechanical stability and integration of the transplant, the maintenance of cellular morphology and function, and the occurrence of degenerative processes. There is no doubt that it is difficult to transfer the model presented here to a clinical application in the light of technical problems, including the supply of epiphyseal chondrocytes as well as conflicting ethical considerations. Nevertheless, the model is suitable for gaining new knowledge regarding the transplantation of proliferating cells and their differentiation in vivo. This is of importance since mesenchymal stem cells, pluripotent periosteal cells, as well as embryonic stem cells, have attracted tremendous interest as cell and tissue replacement strategies, including for the repair of cartilage and bone (52, 53). A growing body of evidence indicates that signals from cell-cell and cell-matrix interactions contribute to cell morphology and the maintenance of the differentiated state (22, 59, 70).

Such interactions are essential for morphogenesis and the development of tissues and organs, as well as for tissue homeostasis. New insights into the cell-to-cell and cell-to-matrix interactions contributing to tissue differentiation and homeostasis (43, 44, 54-56) in these mechanisms will lead to the use of growth and differentiation factors and cells with a defined differentiation potential (mesenchymal stem cells, transplanted cells) as advanced tools for controlled cartilage and bone regeneration.

Acknowledgements

The expert technical assistance of Ms. Melanie Tobler and Ms. Manuela Wiechmann is gratefully acknowledged. We are also grateful to Dr. Hans-Hubert Heilmann for expert advice.

References

20. ROBINSON D, HALPERIN N, NEVO Z: Regenerating hyaline cartilage in articular defects of old chickens using implants of embryonal chick
Chondrocytes in collagen-fibrin-matrices / C. Perka et al.