Tissue Engineered Cartilage Repair Using Cryopreserved and Noncryopreserved Chondrocytes

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The objective of this study was to reconstruct full thickness cartilage defects in rabbit knees with in vitro engineered cartilage tissue based on noncryopreserved and cryopreserved chondrocytes in polymer fleece scaffolds. Osteochondral defects in rabbits were filled with polymer cylinders with noncryopreserved or cryopreserved allogeneic chondrocytes and compared with empty defects and defects filled with polymers alone. The defects were evaluated macroscopically and histologically 4 and 12 weeks after surgery. Transplant samples were graded using a semiquantitative score system. Successful healing was defined as complete integration of a hyalinelike and structurally intact cartilage into the defect and occurred in 71% of the group with noncryopreserved chondrocytes after 4 weeks and 100% of the rabbit knees after 12 weeks, whereas hyalinelike cartilage was seen in 71% of the group with cryopreserved chondrocytes after 4 weeks, and in 85% after 12 weeks. No newly formed cancellous bone was present in the subchondral bone. In the control groups, no cartilagelike tissue was seen. Transplantation of chondrocytes in polymer fleece constructs is a suitable approach for joint cartilage repair. Noncryopreserved chondrocytes are preferred to cryopreserved chondrocytes because of their regenerative potential. In vitro engineered cartilage offers broad opportunities for optimization of cartilage transplantation based on the controlled use of morphogenic and biologically active factors such as transforming growth factor-beta and bone morphogenetic proteins.

List of Abbreviations Used BMP bone morphogenetic protein; TGF- transforming growth factor-beta; APase alkaline phosphatase

A major challenge in this new millennium will be the efficient management and repair of joint cartilage defects. The biologic reconstruction of pathologically altered articular surfaces with cartilage transplants eventually may be an alternative to endoprosthetic arthroplasty.8 The basic principle of the various strategies focused on this objective is the delivery and integration of functionally active cells within an appropriate carrier system into the original tissue to restore the pathologically altered tissue architecture and function. In principle, this procedure initially necessitates amplification of the limited number of cells available and their subsequent phenotypic stabilization in a suitable matrix structure. Biodegradable polymers especially are suited for this purpose because they offer a supportive cellular microenvironment for chondrocytic differentiation.15-17,20-22 In addition, these polymers show a high variability of form and allow specific modification of in vitro graft generation by adding morphogenic factors such as TGF- and BMPs. As a result of its high mechanical stability, the fixation of in vitro preformed tissue at the transplantation site appears to be simple and reliable. In comparison with other carrier substances such as fibrin and collagen, polymers are subject to prolonged degradation, which parallels matrix regeneration.5

However, broad clinical application will require a delay between the day on which autologous cellular material is extracted and the date of retransplantation. Clinical problems and technical reasons for the transplant preparation demand a high degree of flexibility and timing for this kind of treatment, including the supply of allogeneic grafts by cell and tissue banks in the future.

In principle, preservation methods must ensure that the vitality and the phenotypic stability of the cell material are maintained. Although cryopreservation of whole cartilage tissue causes irreversible damage to the chondrocytes,3,10 the synthesis of cartilage specific matrix proteins (collagen Type II, proteoglycans) is maintained over a long period when isolated chondrocytes are cryopreserved.14 After cryopreservation of intact cartilage, the following ultrastructural changes have been reported: the condensation of chromatin, the formation of large lipid drops, partially broken plasma membranes, and the pericellular precipitation of APase positive crystals. After transplantation, cryopreserved osteochondral grafts also showed a clear degeneration of the cartilage portion and marked ultrastructural changes.7,18 However, in vitro studies of cryopreserved chondrocytes have shown reduced mechanical integrity of synthesized cartilage, despite unchanged chondrocyte vitality and proteoglycan synthesis, compared with fresh cells.19 In addition, articular cartilage grafts are subject to immediate mechanical stress. No comparative studies have been done to evaluate the influence of cryopreservation on chondrocyte transplantation.

In the current study, the reconstruction of articular cartilage defects with chondrocytes embedded in a polyglycolic-lactic acid polymer fleece was evaluated. The suitability for initial fixation and subsequent integration of the graft in the defect by means of the synthesized matrix also was studied.

MATERIALS AND METHODS

Twenty-eight 6-month-old male rabbits with 56 defects were included in this study. One defect was made in each knee. After randomization, the rabbits were divided into four groups: two experimental and two control. Fourteen rabbits were euthanized 4 weeks after surgery and 14 rabbits were sacrificed 12 weeks after surgery.

Isolation and Preservation of Chondrocytes

The cartilage of the hips and knees of five 6-month-old rabbits were dissected under sterile conditions and collected in Ham's F-12 medium (Seromed, Berlin, Germany) supplemented with nystatin. The hyaline cartilage was digested by 2 mg/mL collagenase P (Boehringer, Mannheim, Germany) for 12 hours in Ham's F-12 medium supplemented with 10% fetal bovine serum (Seromed), 100 IU/mL penicillin, and 100 g/mL streptomycin. The resulting cell suspension was passed through a 100-m polyester filter (Estal mono, Thal, Switzerland) and washed three times with Ham's F-12 medium (1800 rpm). Isolated cells were divided into two groups: cells of Group 1 (noncryopreserved) were cultured for three passages and subsequently used for transplant preparation, and cells of Group 2 (cryopreservation) were incubated in 10% dimethylsulfoxide, stored at –80°C for 8 weeks, and rapidly thawed and plated for culture thereafter.

Preparation of the Implants

The number of vital cells was determined using a hemocytometer and trypan blue exclusion staining. Cell-fibrin gels were prepared in separate wells of a 24-well tissue plate (Costar, Cambridge, MA). Cells were suspended in Ham's F-12 medium and mixed with the fibrinogen component (Tissucol Duo S-Immuno, Heidelberg, Germany) in a ratio of 3:1. The cell suspension subsequently was seeded into Ethisorb discs with a diameter of 4 mm (Ethicon GmbH, Norderstedt, Germany). A thrombin solution (Tissucol Duo S) in a dilution of 1:10 with phosphate buffered saline was added to the cell-polymer construct to achieve fibrin polymerization. The cell-polymer transplants subsequently were cultured for 2 weeks to allow specific three-dimensional matrix formation in perfusion chambers (Minucells and Minutissue GmbH, Bad Abbach, Germany).[9]

Surgical Procedure

The following standardized surgical procedure was used in all animals. Twenty-eight rabbits had surgery on both knees with one defect made in each knee (total of 56 defects). No animal was excluded from the study because of death, infection, or technical error. The rabbits had surgery under general anesthesia with 0.4 mL/kg Hypnorm (fluanisone 10 mg/mL and fentanyl 0.315 mg/mL; Janssen-Animal Health, Saunderton, United Kingdom) given intramuscularly approximately 25 minutes before surgery. Each knee was exposed by a medial parapatellar incision, and the patella was luxated laterally. An osteochondral defect in the articular cartilage and subchondral bone in the patellar groove (3.5 mm in diameter) was made with a manual drill. The defects were 2.2 mm deep, which was evaluated by the grading of the drill and reflected the thickness of the fleece constructs. Consequently, the top surface of the grafted material was to the level of the original cartilage surface of the joint. The rabbits were divided into four groups of seven defects for each examination period at 4 and 12 weeks, respectively: (1) polyglycolic-lactic acid fleece with noncryopreserved allogeneic chondrocytes; (2) cryopreserved allogeneic chondrocytes; (3) polyglycolic-lactic acid fleece without cells; and (4) untreated defects. The transplants were fixed into the full thickness

defects using fibrin glue (Tissucol Duo S). The patella was reduced and the wound closed in layers with Vicryl (Ethicon).

Histologic and Histochemical Evaluation

The knees of euthanized rabbits were dissected with complete removal of soft tissues. After the macroscopic evaluation, the femoral condyles prepared in this manner were fixed immediately in 4% formaldehyde phosphate buffered saline solutions (pH 7.2) containing 0.5% cetylpyridinium chloride. After the specimens were decalcified with formic acid, they were dehydrated and subsequently embedded in paraffin. The transverse 5-m thick sections through the distal femur were stained with hematoxylin and eosin, Masson-Goldners staining for connective tissue, and Alcian blue (pH 1.0) for sulphated glycosaminoglycan.

Healing Indices

Two persons performed the histologic evaluation of the blinded sections independently in a randomized examination.

All samples were graded with a histologic scale described by Wakitani et al23 and Pineda et al11 (Table 1). Morphologic features of the implant were graded according to five categories: cell morphology, matrix staining, surface regularity, thickness of cartilage, and integration of the transplant.

Category	Points
Cell morphology	
Hyaline cartilage	0
Mostly hyaline cartilage	1
Mostly fibrocartilage	2
Mostly noncartilage	3
Noncartilage only	4
Matrix staining (metachromasia)	
Normal (compared with host adjacent cartilage)	0
Slightly reduced	1
Markedly reduced	2
No metachromatic stain	3
Surface regularity	
Smooth (> ¾)	0
Moderate (> ½–¾)	1
Irregular (¼–½)	2
Severely irregular (< ¼)	3
Thickness of cartilage (relative to host cartilage)	
> ½	0
½–⅓	1
< ⅓	2
Integration of donor with host adjacent cartilage	
Both edges integrated	0
One edge integrated	1
Neither edge integrated	2
Total maximum	14

*Modified according to Pineda et al[11] and Wakitani et al.[23]

TABLE 1. Histologic Grading Scale for the Defects of Cartilage*

Statistical Analysis

Statistical analysis of the scoring results was performed using the Mann-Whitney U test.

RESULTS

Macroscopic Observations

The arthrotomy wound healed completely without complications in all animals within 1 week. All animals could run normally 7 days after the operation. On gross examination, the joints in the experimental groups revealed no signs of osteoarthritis, inflammatory reaction, proliferation of synovium, or cartilage destruction. After 4 weeks the defects were filled completely with smooth white tissue and had acquired a congruent surface with cartilagelike consistency. The original defect was demarcated clearly in all cases after 4 weeks. At 12 weeks the margins of the transplant were barely distinguishable, so the implant appeared to be well integrated into the surrounding normal cartilage. In contrast, animals of the control groups showed signs of cartilage erosion around the defects. The defects of the control group with polymer scaffold alone were almost filled with white shiny tissue with the margins clearly defined. In the control group with empty defects after 4 weeks the defects were filled with dull pale yellow tissue. After 12 weeks, the defects were empty or filled partially with fibrous tissue and had an irregular surface. No differences between the two control groups were observed at this time. The defect area had expanded because of the degeneration of the adjacent cartilage in most cases of the control groups.

Histologic Observations

Noncryopreserved Chondrocytes/Polyglycolic-lactic Acid Polymer Fleece; Cryopreserved Chondrocytes/Polyglycolic-lactic Acid Polymer Fleece

Four weeks after surgery the defects were filled with hyalinelike cartilage tissue. A higher cell density and more uniform cell distribution were seen after transplantation of noncryopreserved cells (Fig 1) leading to slightly better results in the score used according to Pineda et al11 and Wakitani et al.23 This observation was not statistically significant ($p = 0.80$; Table 2). However, in both experimental groups, a high number of small round cells and a decreased matrix to cell ratio compared with normal cartilage were seen. The surface of the patellar groove was restored incompletely. The newly formed cartilage was attached directly to the normal cartilage edge. Compared with normal cartilage, an intense intercellular matrix staining with Alcian blue was visible (Fig 2). Numerous mononuclear cells were observed around the defects filled by polymers with noncryopreserved and cryopreserved chondrocytes. There was no migration of mononuclear cells into the defect because of an inflammatory reaction (Fig 3). The polymers were resorbed partially after 4 weeks. There was no attachment of chondrocytes to the polyglycolic-lactic acid polymer fleece (Fig 4). The scoring results of both experimental groups differed significantly from the results of the control groups after 4 weeks ($p < 0.05$; Table 2).

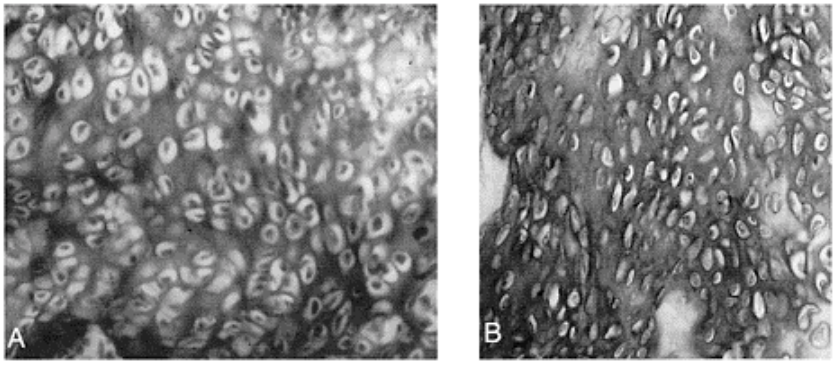

Fig 1A-B. (A) Transverse section of the patellar groove 4 weeks after transplantation of noncryopreserved chondrocytes. A more homogenous cell distribution and higher cell density were observed in the group using noncryopreserved chondrocytes (Stain, Masson-Goldner; original magnification, ¥100). (B) Tissue recovered from a 4-week specimen after transplantation of cryopreserved chondrocytes. The regenerated tissue showed unevenly distributed cells and nonuniform synthesis of glycoaminoglycans compared with noncryopreserved chondrocytes in polyglycolic-lactic acid polymer fleeces (Stain, alcian blue; original magnification, ¥100).

Groups	Weeks After Implantation	Cell Morphology	Matrix Staining	Surface Regularity	Thickness of Cartilage (relative to host cartilage)	Integration of Transplant	Total
Experimental Group I	4	0.7	0.4	1.0	0.8	0.2	3.1
(noncryopreserved chondrocytes)	12	0.8	0.3	0.8	0.4	0.5	2.8
Experimental Group II	4	1.2	0.4	1.5	0.9	0.2	4.2
(cryopreserved chondrocytes)	12	1.0	0.3	1.0	0.6	1.0	3.9
Control Group I	4	3.8	2.4	1.4	2.0	0.2	9.8
(polymer scaffold alone)	12	3.2	2.2	1.6	1.6	0.4	9.0
Control Group II	4	3.8	2.6	2.7	2.0	1.8	12.9
(empty defects)	12	3.1	2.6	2.0	1.6	1.3	10.6

*Modified according to Pineda et al[11] and Wakitani et al.[23]

TABLE 2. Results of the Histologic Grading Score*

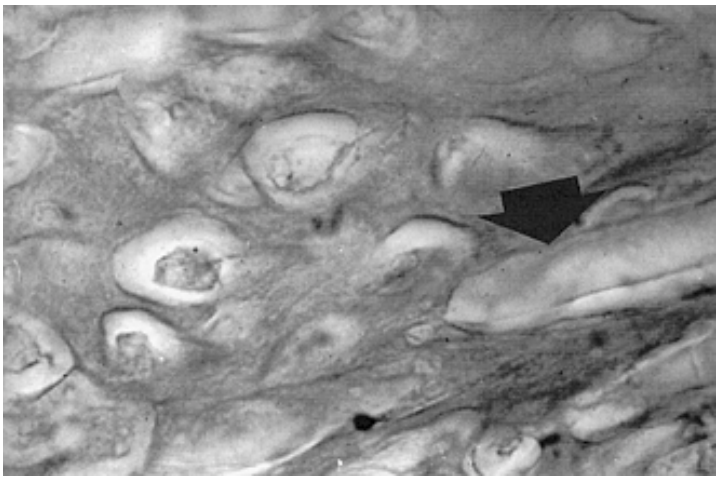

Fig 2. High power view of the regenerated tissue after transplantation of noncryopreserved chondrocytes reveals the normal cellular and matrix characteristics of hyaline articular cartilage. The polymers (arrow) were resorbed partially after 4 weeks (Stain, alcian blue; original magnification, ¥1000).

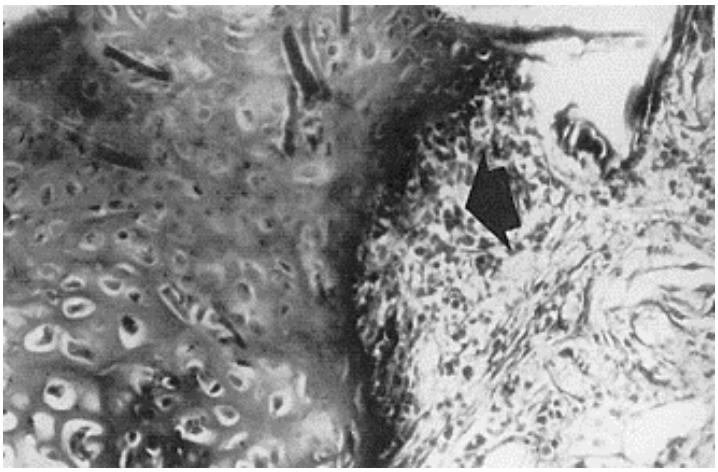

Fig 3. The interface between the newly formed and the original cartilage 4 weeks after transplantation of noncryopreserved chondrocytes. Many mononuclear cells (arrow) are seen around the defect (Stain, Masson-Goldner; original magnification, ¥100).

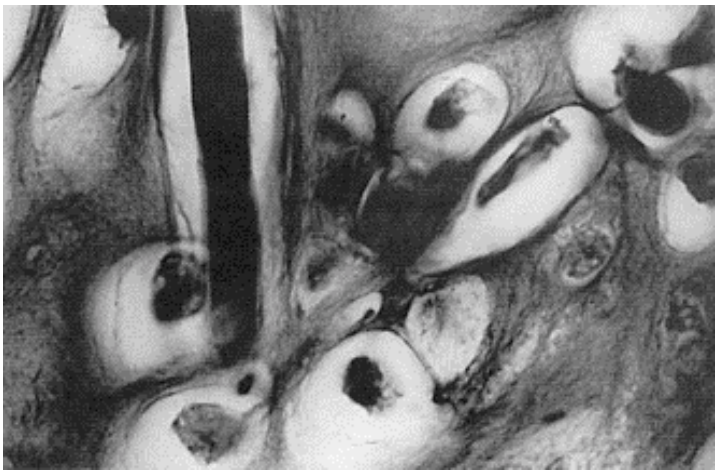

Fig 4. Polymers were resorbed partially after 4 weeks in both cell transplant groups. No attachment of cartilage cells to polymer scaffold was observed (Stain, Masson-Goldner; original magnification, ¥1000).

Twelve weeks after transplantation both experimental groups still showed complete filling with hyalinelike cartilage tissue and direct contact at the defect-cartilage interface (Fig 5). For cryopreserved chondrocytes 12 weeks after transplantation, reduced integrity of newly formed cartilage was evident. The chondrocytes tended to cluster and underwent hypertrophy, especially in the deeper cartilage zones toward the subchondral bone. The superficial cell layers had more spindle-shaped morphologic features, and the surface showed a fibrillar pattern, slightly increased in the group of cryopreserved chondrocytes. Alcian blue staining was less prominent in both groups compared with the evaluation after 4 weeks. With Masson-Goldner staining, a slight pericellular accumulation of collagen was detected. In both groups no necrosis or granulation tissue was seen, and the polymers were resorbed completely. A significantly higher scoring resulted for noncryopreserved chondrocytes in polyglycolic-lactic acid polymer fleeces of Group 1 compared with cryopreserved chondrocytes in polyglycolic-lactic acid polymer fleeces of Group 2 ($p < 0.05$; Table 2). The scoring results of both experimental groups (Groups 1 and 2) differed significantly from results of the control groups (Groups 3 and 4) after 12 weeks ($p < 0.05$, Table 2).

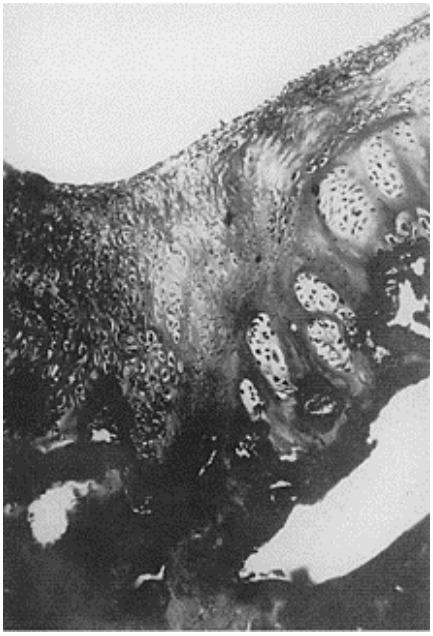

Fig 5. Histologic evaluation 12 weeks after the implantation shows structurally intact cartilage completely filling the defect, but the original articular cartilage architecture is missing (Stain, Masson-Goldner; original magnification, ¥20).

Polyglycolic-lactic Acid Polymer Scaffolds Without Cells; Untreated Defects

There were no apparent differences between the control groups at any time. None of the defects healed with cartilagelike tissue after 4 and 12 weeks. Four weeks after transplantation, soft tissue filled the ground of the defects left empty and connective tissue invaded the polymer (Fig 6). After 12 weeks, the defects left empty contained fibrous scar tissue with abundant spindle-shaped fibroblasts and only a little metaplastic cartilage. The defects filled with polymer had scattered fibrocartilage areas.

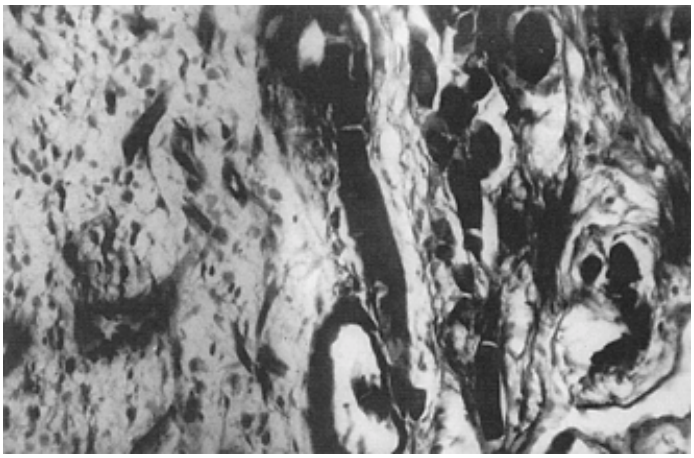

Fig 6. The polymer was almost completely resorbed after 4 weeks (Stain, Masson-Goldner; original magnification, ¥1000).

At all times after transplantation a weaker metachromatic staining with Alcian blue for the control groups compared with the experimental groups was seen. There was no inflammatory reaction. Polymers were resorbed partially after 4 weeks and resorbed completely after 12 weeks. No newly formed cancellous bone reconstructed the subchondral bone.

Success Rate

Successful healing was defined as the formation and complete integration of a hyalinelike and structurally intact cartilage into the host tissue. The success rate of experimental Group 1 (noncryopreserved allogeneic chondrcytes) was 71% (five of seven) after 4 weeks and 100% (seven of seven) after 12 weeks. In Group 2 hyaline cartilage was seen in 71% (five of seven) after 4 weeks and 85% (six of seven) after 12 weeks. In the two control groups, partial fibrocartilage was found after 12 weeks.

DISCUSSION

The cartilage reconstruction of common tissue engineering based approaches demands the interaction of the biodegradable matrix carriers with embedded cells, which maintains their chondrogenic potential and phenotypic differentiation to restore tissue integrity and function.1,2 Resorbable polymer constructs provide a sufficient temporary mechanical stability for the in vitro culture of chondrocytes in a three-dimensional arrangement. After an in vitro culture period of 2 weeks, a cartilaginous tissue of a defined shape was available for subsequent transplantation.22 This applied technique has been used successfully with in vitro engineered cartilage tissue for the repair of rabbit joint defects in short followup. Longer observation time after surgical intervention is necessary to determine the long-term outcome, especially for the evaluation of degenerative changes.

The issue of inhomogeneous cell distribution within the fiber mesh and cell attachment to the fiber structures was addressed as a problem for chondrocyte transplantation in polymer structures in another study.4 In the current investigation, suspension of chondrocytes in fibrinogen and subsequent polymerization of this fibrin gel within the polyglycolic-lactic acid polymer fleece seemed to be an efficient way to overcome this crucial limitation. Cells crosslinked by fibrin within biocompatible resorbable polymers improve malleability and shaping of the transplants. In contrast to chondrocyte transplantation techniques without carrier systems, this combination is advantageous for resurfacing irregular and large cartilage defects. In terms of transplant fixation the procedure applied was simple and safe. In contrast to other authors,12 who described frequent dissolution of the gel-like matrix substance used, there were no cases of detachment of the transplant, although it was stressed mechanically immediately from the first post-operative day.

In addition, the rate of polyglycolic-lactic acid polymer degradation was slow enough to ensure stable integration of the transplant through a sufficient synthesis of cartilage-specific matrix proteins. Four weeks after implantation, remnants of the fiber structures were detectable in all cases; however, after 12 weeks they were resorbed completely. As a result of the degradation of the polymer, a mild inflammatory reaction with an accumulation of mononuclear cells around the defect was seen 4 weeks after transplantation. However, cell migration into the defect was not found in any of the cases. Twelve weeks after surgery, inflammatory cells no longer were observed around the defect, which may be attributable to the polymers having been resorbed completely by this time. Neither the implantation of polymers alone nor empty defects revealed the formation of hyaline cartilage. The defect always was repaired with fibrous cartilage only. This repair was a result of the migration of mesenchymal cells into the defect, which led to reorganization of functionally inferior tissue in untreated defects and after the transplantation of polymers only. The fibrous cartilage formed is known to be unstable and result in progressive degeneration.14 Both groups showed comparable results in the semiquantitative assessment, although structurally better filling of the defect was observed after transplantation of polymers. The scaffolds presumably

functioned as guiding structures for host mesenchymal cells migrating into the defect and regenerating the cartilage matrix.6

Consequences from this observation are not yet known; the incorporation of growth and differentiation factors such as BMPs into the polymer scaffolds and their slow release may have a crucial impact on tissue reconstitution. Defect healing with hyaline cartilage was observed only in the experimental groups, with a success rate to 100% using noncryopreserved chondrocytes after 12 weeks after surgery. The results of the 4-week group slightly differed from the results reported by Freed et al4 in which no significant differences were found between cell-polymer transplants and polymer transplants alone after 4 weeks after surgery with polyglycolic acid polymer chondrocyte transplants. This may be because of the different polymer structure and the described modifications with fibrin crosslinked cells.

Another question addressed in the current study was the feasibility of cryopreserved chondrocytes for tissue engineering based transplant procedures. The semiquantitative assessment of histologic features according to the score of Pineda et al11 showed that there was a tendency toward poorer results with transplantation of cryopreserved chondrocytes. This was revealed by the lower incidence of cartilage-specific cell morphologic features after 4 weeks and inferior defect integration 12 weeks after surgery. Reasons for this observation may be a higher incidence of irreversibly damaged cryopreserved chondrocytes still vital at the time of transplantation. It appears that the transplantation of noncryopreserved chondrocytes is preferred over cryopreserved cells. Long-term studies are needed to confirm that the results obtained with the grading score used reflect biologically relevant morphologic differences. However, healing can be achieved with hyalinelike cartilage, even after transplantation of cryopreserved cells in most cases.

Engineering of smart polymers tailored for specific requirements offers new perspectives for tissue regeneration and repair. The design flexibility of the biodegradable scaffolds allows modification with specific extracellular matrix components (hyaluronic acid) or adhesion molecules to provide a supportive microenvironment for the embedded cells and cells of the neighboring tissue. The investigation of the pathogenesis of osteoarthritis will have a major impact on cartilage engineering: bioactive molecules such as cytokines, inhibitors of proteases, or morphogenic proteins locally released from the scaffold structures or by genetically modified cells may counteract the process of matrix depletion and degradation. In addition, the development of new composite grafts with bone and cartilage components leads to new avenues in the reconstruction of full thickness cartilage defects. Advanced tissue engineering approaches focus on the controlled use of mechanical stimuli during the in vitro stage of transplant preparation to enhance biomechanical stability.

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References

1. Benya PD, Shaffer JD: Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 30:215-224, 1982.

2. Bonaventure J, Kadhom N, Cohen Solal L, et al: Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. Exp Cell Res 212:97-104, 1994.

3. Bujia J, Kremer D, Sudhoff H, et al: Determination of viability of cryopreserved cartilage grafts. Eur Arch Otorhinolaryngol 252:30-34, 1995.

4. Freed LE, Grande DA, Lingbin Z, et al: Joint resurfacing using allograft chondrocytes and synthetic biodegradable polymer scaffolds. J Biomed Mater Res 28:891-899, 1994.

5. Freed LE, Vunjak-Novakovic G, Langer R: Cultivation of cell-polymer cartilage implants in bioreactors. J Cell Biochem 51:257-264, 1993.

6. Hunziker EB, Rosenberg L: Induction of repair in partial thickness articular cartilage lesions by timed release of TGF-. Trans Orthop Res Soc 19:236, 1994.

7. Malinin TI, Mnaymneh W, Lo HK, Hinkle DK: Cryopreservation of articular cartilage. Ultrastructural observations and long-term results of experimental distal femoral transplantation. Clin Orthop 303:18-32, 1994.

8. Mankin HJ, Fogelson FS, Thrasher AZ, Jaffer F: Massive resection and allograft transplantation in the treatment of malignant bone tumors. N Engl J Med 294:1247-1255, 1976.

9. Minuth WW, Kloth S, Aigner J, Sittinger M, Röckl W: Approach to an organo-typical environment for cultured cells and tissues. Biotechniques 20:498-501, 1996.

10. Ohlendorf C, Tomford WW, Mankin HJ: Chondrocyte survival in cryopreserved osteochondral articular cartilage. J Orthop Res 14:413-416, 1996.

11. Pineda S, Pollack A, Stevenson S, Goldberg V, Caplan A: A semiquantitative scale for histologic grading of articular cartilage repair. Acta Anat Basel 143:335-340, 1992.

12. Rahfoth B, Weisser J, Sternkopf F, et al: Transplantation of allograft chondrocytes embedded in agarose gel into cartilage defects of rabbits. Osteoarthritis Cartilage 6:50-65, 1998.

13. Schachar N, Nagao M, Matsuyama T, McAllister D, Ishii S: Cryopreserved articular chondrocytes grow in culture, maintain cartilage phenotype, and synthesize matrix components. J Orthop Res 7:344-351, 1989.

14. Shapiro F, Koide S, Glimcher MJ: Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg 75A:532-553, 1993.

15. Sittinger M, Bujia J, Hammer C, Minuth WW, Burmester GR: Engineering of cartilage tissue using bioresorbable polymer carriers in perfusion culture. Biomaterials 15:451-456, 1994.

16. Sittinger M, Reitzel D, Dauner M, et al: Resorbable polyesters in cartilage engineering: Affinity and biocompatibility of polymer fibre structures to chondrocytes. J Biomed Mater Res 33:57-63, 1996.

17. Sittinger M, Schultz O, Keyszer G, Minuth WW, Burmester GR: Artificial tissues in perfusion culture. Int J Artif Organs 20:57-62, 1997.

18. Tavakol K, Miller RG, Bazett Jones DP, et al: Ultrastructural changes of articular cartilage chondrocytes associated with freeze-thawing. J Orthop Res 11:1-9, 1993.

19. Thomas VJ, Jimenez SA, Brighton CT, Brown N: Sequential changes in the mechanical properties of viable articular cartilage stored in vitro. J Orthop Res 2:55-60, 1984.

20. Vacanti CA, Cao YL, Upton J, Vacanti JP: Neo-cartilage generated from chondrocytes isolated from 100-year-old human cartilage. Transplant Proc 26:3434-3435, 1994.

21. Vacanti CA, Kim W, Schloo B, Upton J, Vacanti JP: Joint resurfacing with cartilage grown in situ from cell-polymer structures. Am J Sports Med 22:485-488, 1994.

22. Vacanti CA, Upton J: Tissue-engineered morphogenesis of cartilage and bone by means of cell transplantation using synthetic biodegradable polymer matrices. Clin Plast Surg 21:445-462, 1994.

23. Wakitani S, Kimura T, Hirooka A, et al: Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg 71B:74-80, 1989.

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