Formation of Cartilage Repair Tissue in Articular Cartilage Defects Pretreated with Microfracture and Covered with Cell-Free Polymer-Based Implants

Christoph Erggelet,[1] Michaela Endres,[2,3] Katja Neumann,[2] Lars Morawietz,[4] Jochen Ringe,[5] Kathrin Haberstroh,[3] Michael Sittinger,[3] Christian Kaps[2,3]

[1]Department of Traumatology and Orthopaedic Surgery, University of Freiburg, Hugstetter Str. 55, 79106 Freiburg, Germany, [2]TransTissue Technologies GmbH, Tucholskystrasse 2, 10117 Berlin, Germany, [3]Tissue Engineering Laboratory, Department of Rheumatology, Charité Campus Mitte, Charité—Universitätsmedizin Berlin, Tucholskystrasse 2, 10117 Berlin, Germany, [4]Institute of Pathology, Charité Campus Mitte, Charité—Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany, [5]Berlin–Brandenburg Center for Regenerative Therapies, Charité—Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

Received 2 December 2008; accepted 11 February 2009
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20879

ABSTRACT: The aim of our study was to evaluate the mid-term outcome of a cell-free polymer-based cartilage repair approach in a sheep cartilage defect model in comparison to microfracture treatment. Cell-free, freeze-dried implants (chondrotissue®) made of a poly-glycolic acid (PGA) scaffold and hyaluronan were immersed in autologous serum and used for covering microfractured full-thickness articular cartilage defects of the sheep ($n = 4$). Defects treated with microfracture only served as controls ($n = 4$). Six months after implantation, cartilage implants and controls were analyzed by immunohistochemical staining of type II collagen, histological staining of proteoglycans, and histological scoring. Histological analysis showed the formation of a cartilaginous repair tissue rich in proteoglycans. Histological scoring documented significant improvement of repair tissue formation when the defects were covered with the cell-free implant, compared to controls treated with microfracture. Immunohistochemistry showed that the cell-free implant induced cartilaginous repair tissue and type II collagen. Controls treated with microfracture showed marginal formation of a mixed-type repair tissue consisting of cartilaginous tissue and fibro-cartilage. Covering of microfractured defects with the cell-free polymer-based cartilage implant is suggested to be a promising treatment option for cartilage defects and improves the regeneration of articular cartilage.

Keywords: cartilage repair; cartilage regeneration; cell-free implant; microfracture; stem cells

Injuries of the articular cartilage of the knee are a common challenge in orthopedic surgery. The low inherent regeneration capacity of articular cartilage and the risk of potentially developing severe osteoarthritis from injured cartilage led to the development of a variety of orthopedic repair techniques. Common cartilage repair techniques comprise debridement, bone marrow stimulating techniques, osteochondral grafting, and autologous chondrocyte implantation.[1–4] Autologous chondrocyte implantation (ACI) has been shown to be clinically effective when implanting culture-expanded chondrocytes alone[5–8] or in combination with resorbable scaffolds made of collagen, hyaluronan, or polymers.[9–11]

However, in clinical routine, bone marrow stimulating techniques like drilling, abrasion, or microfracture are frequently used,[1,12,13] are cost effective, and are first-line treatment options for focal cartilage defects. In microfracture, the introduction of multiple perforations into the subchondral bone of the cartilage defect leads to bleeding, allows mesenchymal stem and progenitor cells from the bone marrow to enter the defect, and induces formation of cartilaginous repair tissue. The cellular mechanisms underlying stem cell migration into the defect and subsequent tissue formation are not obvious. Recently, it has been shown that synovial fluid recruits mesenchymal progenitor cells from bone marrow.[14] In addition, a variety of chemotactic cytokines and growth factors, components of synovial fluid and serum, stimulates migration and homing of mesenchymal stem cells[15,16] and may contribute to ingrowth of progenitors into the cartilage defects in microfracture. Multi-potent cells residing in the subchondral cortico-spongious bone marrow have a high chondrogenic differentiation capacity[17] and may form a cartilaginous repair tissue upon stimulation by growth and differentiation factors from the subchondral bone.[18,19] Although clinical studies demonstrated that microfracture shows good results in the short term,[6,20] the repair tissue induced by microfractures has been shown to be of a hyaline to fibrous appearance with limited short-term durability. However, long-term studies with up to 17 years follow-up showed that 80% of the patients treated with microfracture improved compared to the preoperative situation, while 20% showed no improvement or considered the pain worse postoperatively.[21] Interestingly, in a group of 85 patients with full-thickness cartilage defects, the clinical situation improved in the short-term at 18 months follow-up. In the mid- to long-term, the clinical scores significantly decreased at 36 months compared to 18 months follow-up, but were significantly increased compared to the preoperative situation.[22] Obviously, the microfracture treatment shows good short-terms results, but clinical results may be variable in the long-term. In addition, the microfracture technique may be limited by its indication for relatively small defects and the need for an intact defect

Michaela Endres and Katja Neumann contributed equally to this work.

Correspondence to: C. Kaps (T: ++49-(0)30-450-513293; F: ++49-(0)30-450-513957; E-mail: christian.kaps@charite.de)

shoulder surrounding the defect. Therefore, further development of the microfracture technique is indicated that may improve cartilaginous repair tissue formation by, for instance, enhancing the content of key cartilage matrix components[23] and by covering the defects as well as by extending the indication to isolated defects with, at least, a partly missing defect shoulder.

Recently, we have shown that mesenchymal progenitors are recruited by autologous serum and that hyaluronan supports the chondrogenic differentiation of human mesenchymal progenitors. The cell-free implant made from a polyglycolic acid scaffold, hyaluronan and serum improved cartilage repair in ovine cartilage defects pretreated with microfracture in the short-term with 3 months follow-up.[24] We hypothesize that covering of cartilage defects with a polymer-based cell-free implant after microfracture improves microfracture-induced cartilage repair and leads to a more hyaline-like repair tissue rich in type II collagen compared to microfracture treatment alone in the mid-term at 6 months follow-up.

MATERIALS AND METHODS

Implantation of Cell-Free Implants in Cartilage Defects of the Sheep

The cell-free implant was manufactured under aseptic conditions. The resorbable scaffold ($15 \times 20 \times 1.1$ mm^3) of pure polyglycolic acid (Alpha Research Deutschland GmbH, Berlin, Germany) was immersed in 330 µl hyaluronic acid (10 mg/ml Ostenil®, TRB Chemedica AG, München, Germany) as described previously.[24] Implants were freeze-dried for 16 h using a lyophilizator (Leybold-Heraeus, Köln, Germany) and stored in a desiccator at room temperature.

The study has been approved by the Review Board for the Care of Animal Subjects at the Regierungspräsidium Freiburg, Germany. Eight adult Merino sheep (female; age: 3 years) were used in this study. All surgical procedures were performed under general anesthesia (isoflurane inhalation) and under aseptic conditions. The medial femoral condyle of the left stifle joint was exposed using a medial approach. Degeneration of the joint and skeletal abnormalities were excluded by visual inspection and full-thickness cartilage defects of 11×8 mm were created in the weight-bearing cartilage using a scalpel and a curette. Depending on the size of the sheep, the cartilage surface of the medial femoral condyle is about 5 cm^2 with a less load-bearing rim of 2–3 mm. A defect of 11×8 mm corresponds to approximately 20%–25% of the load-bearing area of the medial femoral condyle. In every defect, nine microfracture perforations were introduced using a chondropick®, until bleeding was observed. Autologous sheep serum was obtained intraoperatively using standard serum monovettes (Sarstedt, Nümbrecht, Germany). The cell-free cartilage implants ($11 \times 8 \times 1.1$ mm) were immersed in the sheep serum for 10 min, used for covering of the defects ($n = 4$), and securely fixed trans-osseously as described.[25] Cartilage defects with microfracture perforations but without covering with cell-free implants served as controls ($n = 4$). Sheep were allowed to recover in boxes for 10 days with full load-bearing and were kept out at feed thereafter. At 6 months, sheep were killed by injection of an overdose of thiopentone followed by potassium chloride, intravenously.

Histology and Immunohistochemistry

For histological and immunohistochemical analyses, joints were fixed in 4% buffered formalin, decalcified with EDTA for 4 weeks, embedded in paraffin, and microsectioned at 6 µm. Proteoglycans were visualized by staining with Alcian Blue 8GS (Roth, Karlsruhe, Germany) at pH 2.5, followed by counterstaining with nuclear fast red (Sigma, St. Louis, MO). For histological scoring, sections were stained with hematoxylin-eosin and sections through the center of the defect ($n = 2$ per specimen, $n = 8$ per group) were evaluated by the scoring systems according to O'Driscoll et al.,[26] Pineda et al.,[27] and Wakitani et al.[28] A low score (min. 0; max. 15), according to the Wakitani as well as the Pineda scoring system, describes a native-like cartilage tissue, a good defect filling, and good integration into the surrounding host tissue. The score according to O'Driscoll is inverted with a high score (min. 0; max. 24) representing native-like cartilage repair tissue. Histological scoring was performed by two independent observers and the mean was calculated. The t-test was applied for statistical evaluation and differences were considered significant when $p < 0.05$.

For immunohistochemical analysis of type II collagen, sections were digested for 30 min with hyaluronidase (100 U/ml in PBS) and fixed in ice-cold methanol and acetone. Sections were incubated for 1 h with primary antibodies (rabbit anti-bovine type II collagen, Biologo, Kronshagen, Germany) or rabbit IgG as control (DAKO, Hamburg, Germany). Subsequently, sections were processed using the EnVision System Peroxidase Kit (DAKO, Hamburg, Germany) according to the manufacturer's instructions, followed by counterstaining with hematoxylin (Merck, Darmstadt, Germany). Controls gave no signal.

RESULTS

Clinical and Gross Examination

At 6 months, all sheep were out at feed and showed no lameness or abnormal behavior. There were no clinical signs of inflammation, infection, or allergic reaction. The exposed joints showed no signs of synovial inflammation or irritation. The synovial fluid was clear and appeared to be normal. A complete reconstruction of the articular cartilage and the surface as well as complete filling of the defects were not evident.

Histological Evaluation of Full-Thickness Cartilage Defects Treated with Microfracture

Histological analysis of control defects treated with microfracture was performed with emphasis on central regions of the defects with cartilaginous repair tissue. Staining of proteoglycans with Alcian blue is shown for each individual defect (Fig. 1). After 6 months, the defects treated with microfracture without covering with the cell-free implant showed formation of marginal and nodule-like repair tissue. In one case, the subchondral bone plate was broken, collapsed, and showed pronounced ingrowth of fibrous and granular tissue void of proteoglycans (Fig. 1A, black arrowhead), and formation of vascularized scar tissue (Fig. 1A, white arrowheads). Intense remodeling of the subchondral bone was observed (Fig. 1A, asterisk; Fig. 1B, black arrowhead) and proteoglycan-rich repair tissue was evident at the level of the subchondral bone plate

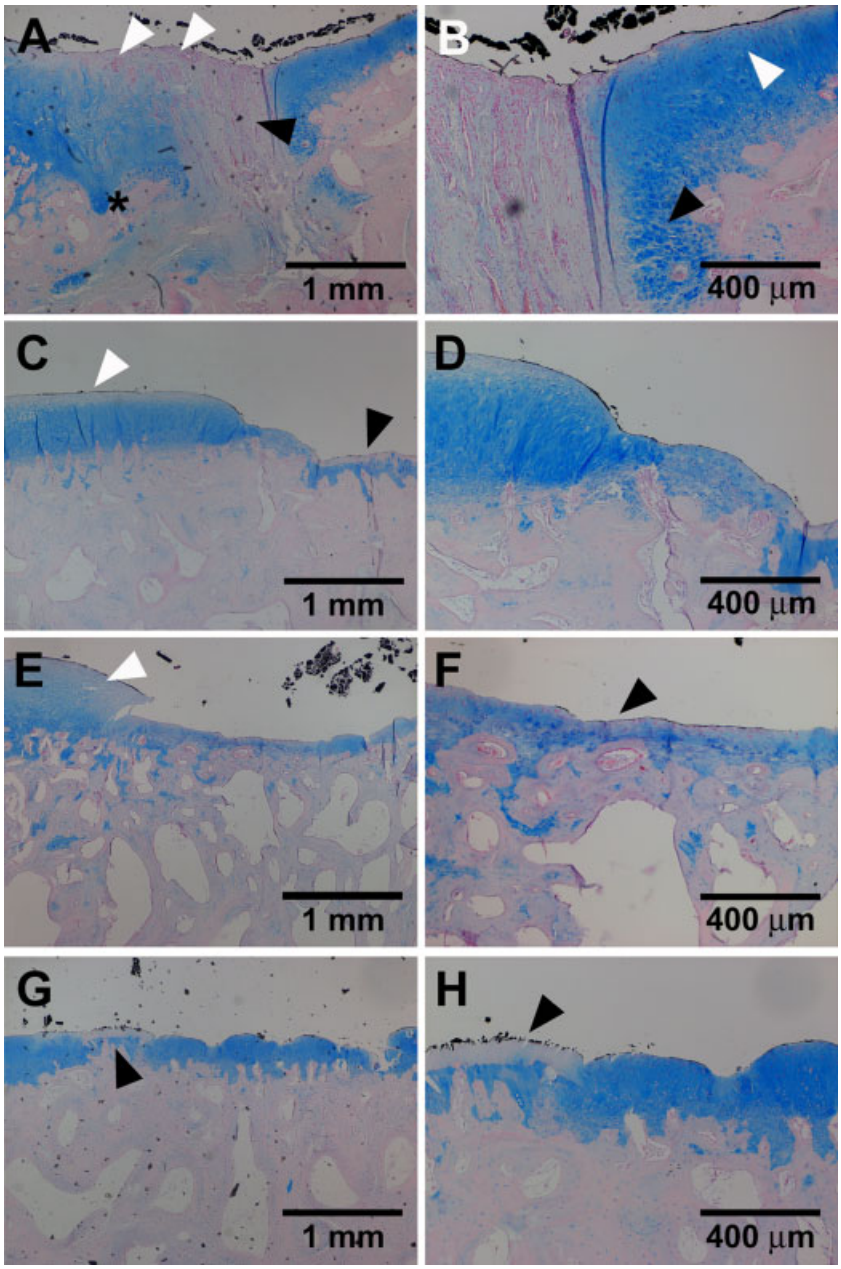

Figure 1. Histological staining of proteoglycans in repair tissue formed after microfracture. At 6 months, Alcian blue staining was variable in repair tissue formed after microfracture treatment in control defects. One of the defects (A) showed a fibrous, granular scar-like tissue with vascular ingrowth (A, white arrowheads) void of proteoglycans (A, black arrowhead) and extensive remodeling of the subchondral bone (A, asterisk). Adjacent to the fibrous tissue, proteoglycan-rich repair tissue (B, white arrowhead) and subchondral bone remodeling (B, black arrowhead) was observed. Next to the repair tissue that was rich in proteoglycan (C, white arrowhead; D), there was subchondral bone showing no repair tissue formation (C, black arrowhead). Another defect showed nodule-like repair tissue formation (E, white arrowhead) and a thin cell layer covering the subchondral bone plate (F, black arrowhead). Incidentally, remnants of the original cartilage (G, H, black arrowhead) were evident.

(Fig. 1B, white arrowhead). Adjacent to regions with proteoglycan-rich cartilaginous repair tissue (Fig. 1C/D), subchondral bone with no signs of repair tissue was evident (Fig. 1C, black arrowhead). The regions with repair tissue were irregular and showed nodule-like cartilaginous tissue (Fig. 1E, white arrowhead) and marginal tissue formation with a thin cell layer (Fig. 1F, black arrowhead). In some regions, incidental remains of the debrided articular cartilage were evident and the repair tissue achieved the level of the subchondral bone plate (Fig. 1G/H, black arrowhead).

The quality of the repair tissue formed in control defects after microfracture was assessed by immunohistochemical staining of type II collagen (Fig. 2). The presence of type II collagen in the repair tissue formed after microfracture treatment was variable. Some regions with repair tissue showed an intense staining of type II collagen (Fig. 2A), while others showed no (Fig. 2B) or marginal staining of type II collagen (Fig. 2C). Morphologically, the cells were either of a chondrocyte-type or stretched and of a fibroblastoid phenotype scattered across the repair tissue (Fig. 2B, D, white arrowhead).

Histological Evaluation of Full-Thickness Cartilage Defects Pretreated with Microfracture and Covered with the Cell-Free Implant

Histological analysis of the repair tissue in defects after microfracture treatment and implantation of the cell-free implant showed the formation of cartilaginous tissue with intense staining of proteoglycans (Fig. 3A–F). The repair tissue was void of polymer

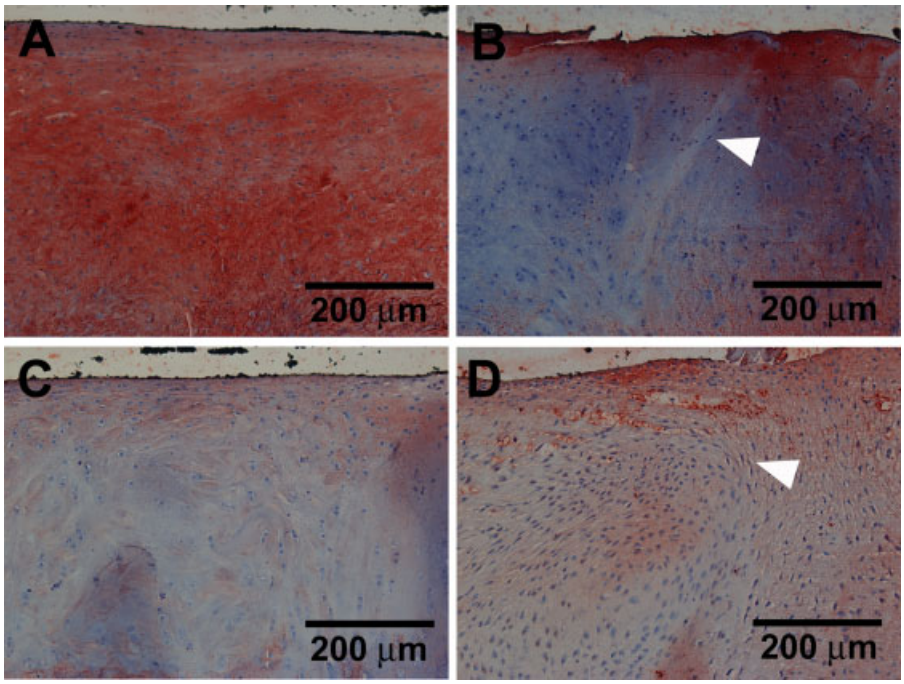

Figure 2. Immunohistochemical staining of type II collagen in repair tissue formed after microfracture. At 6 months, the repair tissue formed after microfracture treatment in control defects showed a variable presence of type II collagen with an intense (A), marginal (B), and moderate (C, D) staining of the cartilage matrix component. The repair tissue showed a mixed cell population of a round-shaped and fibroblastoid phenotype (B, D, white arrowhead).

fibers and rich in viable round-shaped cells that were evenly distributed across the cartilaginous tissue. In some regions, a columnar and chondron-like distribution of cells with some clustering was found (Fig. 3F, white arrowhead). Signs of abnormal calcification, infiltration of immunological cells, apoptosis of cells, or necrosis within the repair tissue were not evident. The surface of the repair tissue developed after microfracture treatment, and covering with the cell-free implant was regular and smooth (Fig. 3A, C, E, white arrowheads). The repair tissue developed in one out of four defects was rich in cells and showed a variable staining with regions showing no (Fig. 3G, black arrowhead) to weak staining of proteoglycans (Fig. 3H, white arrowhead). The cell-rich cartilaginous repair tissue showed good bonding to the subjacent subchondral bone that still underwent remodeling.

Immunohistochemical staining showed that cartilage-specific type II collagen was present in repair tissue developed after pretreatment of cartilage defects with microfracture and implantation of the cell-free polymer-based implant (Fig. 4). Type II collagen staining showed a dominant to medium intensity in the repair tissue of each individual defect (Fig. 4A–D). The staining indicated an even distribution of cartilage matrix components within the repair tissue, with type II collagen in regions contiguous to the subchondral bone plate and towards the joint space.

Histological Scoring

For quantitative evaluation of repair tissue formation after microfracture compared to defects pretreated with microfracture and covered with the cell-free-implant, histological scores were applied (Fig. 5). Defects treated with microfracture were rated after 6 months with a histological score of 9.5 (Wakitani, Fig. 5A), 9.0 (Pineda, Fig. 5B), and 7.8 (O'Driscoll, Fig. 5C). Covering the defects pretreated with microfracture with the cell-free implant significantly ($p < 0.0075$) improved cartilaginous repair tissue formation. Defects that received the implant were scored with 5.1 (Wakitani), 5.0 (Pineda), and 14.7 (O'Driscoll).

DISCUSSION

In the present study, we demonstrated that treatment of full-thickness ovine cartilage defects with microfracture and covering with a cell-free cartilage implant made of a poly-glycolic acid scaffold, hyaluronan and autologous serum improved cartilage repair tissue formation compared to microfracture treatment alone in the mid-term outcome after 6 months. The repair tissue formed in the defects showed abundant amounts of proteoglycans and type II collagen suggesting the formation of a cartilaginous to hyaline-like tissue after implantation of the cell-free implant. Defects treated with microfracture alone formed a mixed, cartilaginous to fibrous tissue with variable amounts of cartilage-specific type II collagen.

Clinically, the microfracture technique is a frequently used, first-line cartilage repair option and induces the formation of cartilage repair tissue by perforating the subchondral bone. These microfractures allow mesenchymal progenitor cells to populate the defect and form cartilaginous tissue.[1] However, the repair tissue that is induced by microfracture may appear unstructured and shows predominantly fibro-cartilage. For instance, a recent clinical study in young athletes with a 3-year follow-up showed that fibro-cartilage and surface fibrillation were evident in 8 out of 14 biopsies.[29] This is consistent with the data presented here using the ovine

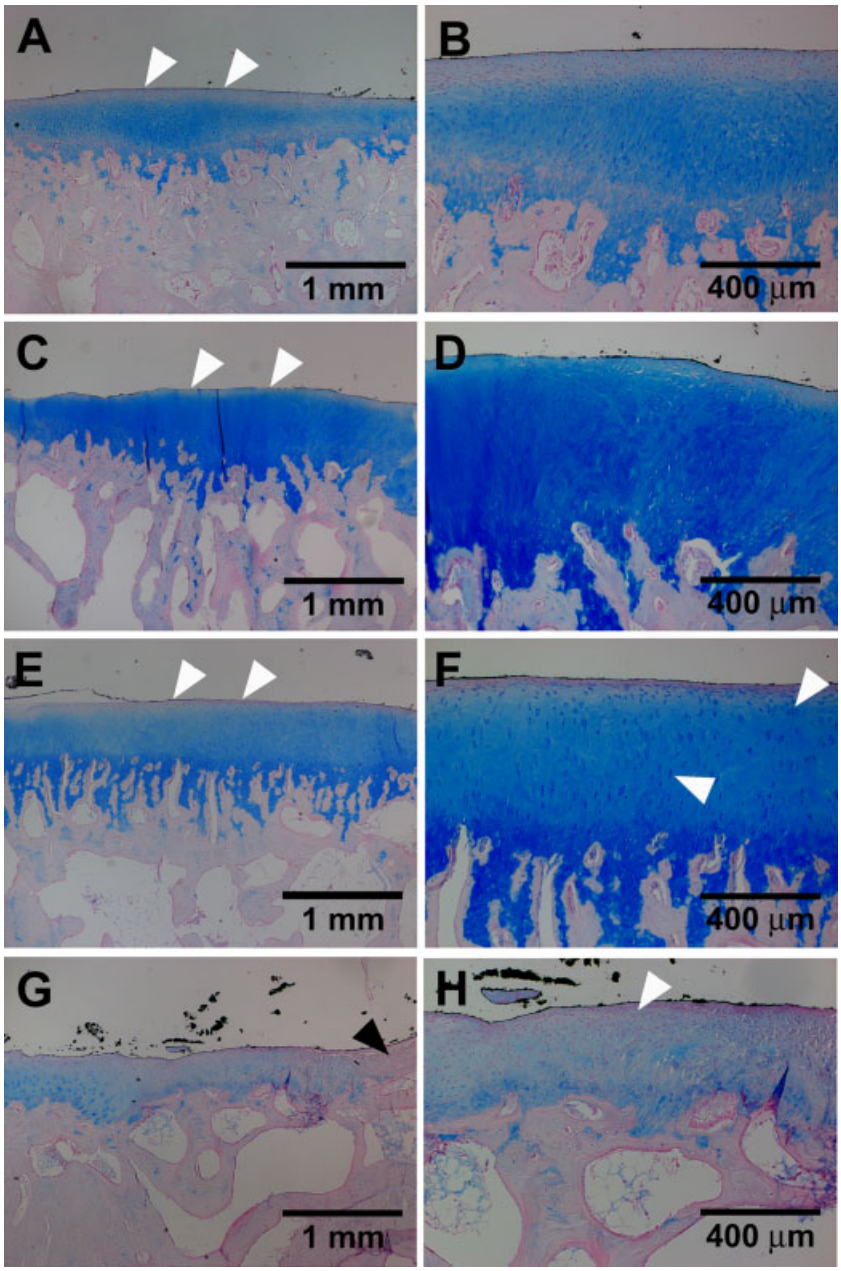

Figure 3. Histological staining of proteoglycans in repair tissue formed in defects pretreated with microfracture and covered with the cell-free implant. At 6 months, formation of a cartilaginous repair tissue was evident that showed intense staining of proteoglycans as assessed by Alcian blue staining (A–F). The surface of the repair tissue formed in the defect was even and smooth (A, C, E, white arrowheads). The cartilaginous repair tissue was rich in viable cells of a round-shaped phenotype and showed some columnar cell distribution (F, white arrowheads). In one of the defects, the repair tissue was variable with a faint staining of proteoglycans and regions void of proteoglycans (G, black arrowhead). The repair tissue with moderate formation of a cartilaginous extracellular matrix showed vital round-shaped cells of a chondrocytic phenotype (H, white arrowhead).

cartilage defect model. In the mid-term outcome, defects treated with microfracture formed a repair tissue that was of fibrous to cartilaginous appearance with a variable presence of cartilage-related type II collagen. Recently, we showed that human serum recruits mesenchymal progenitors, that hyaluronan supports cartilage matrix formation, and that covering of ovine defects pretreated with microfracture enhances cartilage repair in the short-term.[24] This suggests that covering of the defects treated with microfracture with the polymer-based implant may accelerate repair tissue formation, may enhance cartilage-related repair tissue composition, and may improve cartilage regeneration compared to microfracture treatment.

In microfracture, it is suggested that vaso-active factors, growth factors and cytokines that are released by platelets, washed into the defect by bleeding or released from subchondral microfractures may influence the recruitment of mesenchymal progenitors and subsequent cell differentiation and tissue development.[19,30] Migration of cells into, or enrichment of, progenitors within the cell-free implant may be induced by autologous serum that contains a variety of chemokines and growth factors. In recent in vitro studies, it has been shown that chemokines and growth factors,[16,31] as well as human synovial fluid[14] and human serum,[24] are potent inducers of mesenchymal stem cell migration. In particular, human serum and blood may be of special interest in cartilage repair, since blood has been shown to improve hyaline cartilage formation after microfracture in a rabbit model when combined with a chitosan-glycerol-phosphate implant.[32] Therefore, as used here

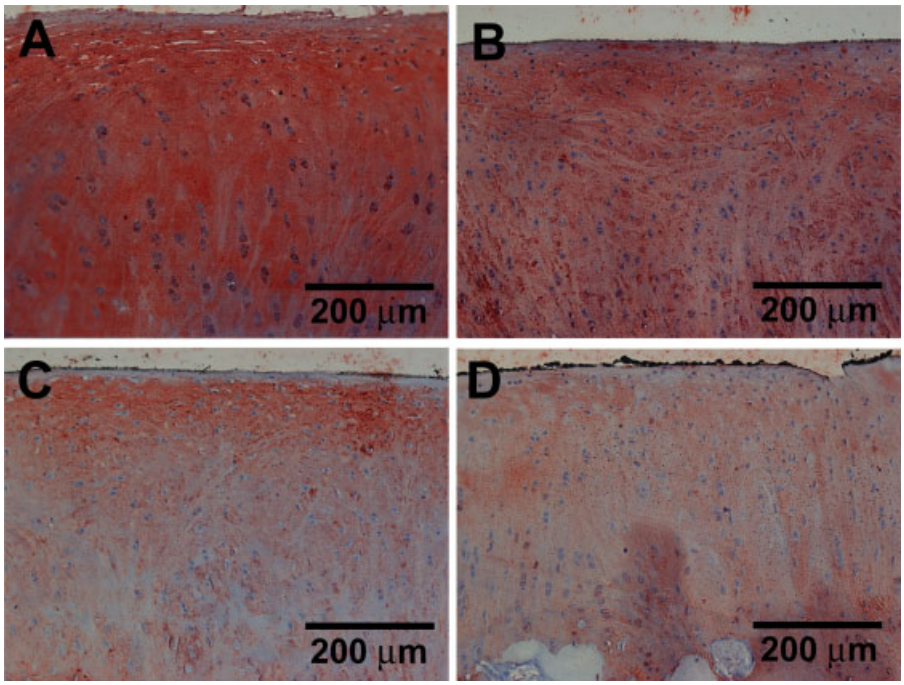

Figure 4. Immunohistochemical staining of type II collagen in repair tissue formed in defects pretreated with microfracture and covered with the cell-free implant. At 6 months, the repair tissue that formed after microfracture treatment and covering with the cell-free implant was of a hyaline-like to hyaline appearance as shown by the presence of type II collagen. The repair tissue showed abundant amounts of the cartilage-related collagen with an intense (A, B) to moderate (C, D) staining of type II collagen.

in microfracture, serum may support the enrichment of multi-potent mesenchymal progenitor cells within the cartilage defect and support hyaline repair tissue formation.

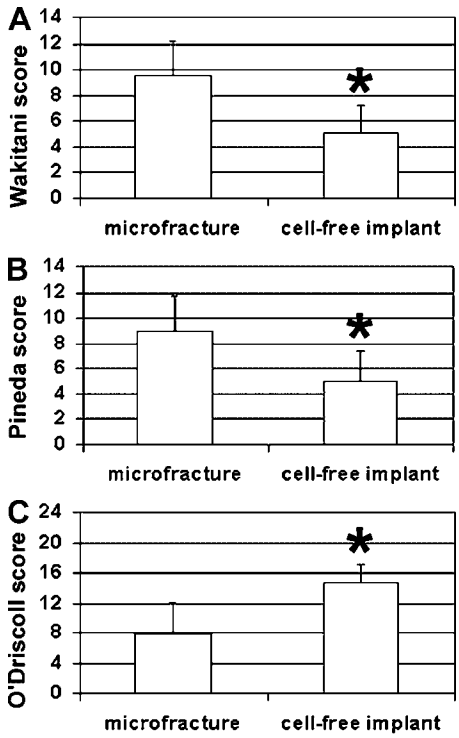

Figure 5. Quantitative evaluation of repair tissue formation. At 6 months after treatment of the defects, histological scoring according to Wakitani (A), Pineda (B), and O'Driscoll (C) showed that covering of defects pretreated with microfracture with a cell-free implant based on the poly-glycolic acid scaffold, hyaluronan and autologous serum significantly (*, $p < 0.0075$) improved cartilage repair tissue formation compared to microfracture treatment alone.

Subchondral cortico-spongious bone harbors multipotent mesenchymal progenitors with a high chondrogenic potential.[17] Chondrogenic differentiation of mesenchymal progenitors can be induced by stimulation of cells with, e.g., all isoforms of transforming growth factor and selected bone morphogenetic proteins in micromasses.[33–35] In addition, synovial fluid and hyaluronan (HA), a key component of the synovial fluid, have been shown to induce the chondrogenic developmental sequence with deposition of proteoglycan and type II collagen in equine mesenchymal stem cells derived from bone marrow.[36] In microfracture, the application of a HA gel resulted in improved cartilage regeneration with thicker cartilage and more hyaline-like cartilage repair tissue in a rabbit model.[37] In addition, HA has been shown to support the chondrogenic development as shown by the induction of type II collagen and repression of type I collagen of human mesenchymal stromal cells stimulated with transforming growth factor (TGF) in a HA scaffold.[38] These reports suggest that hyaluronic acid may induce or, at least, support the chondrogenic development of mesenchymal progenitors in microfracture.

In our cell-free cartilage regeneration approach, the implant made of a textile polymer-based scaffold, hyaluronan and autologous serum improved repair of full-thickness cartilage defects in microfracture. However, limitations of the study are the limited number of sheep and the lack of a correlation between different defect sizes and the "regenerative success" of microfracture treatment with and without covering by the polymer scaffold. This may help to decide whether a given defect can be treated with microfracture only or should be microfractured and covered with a resorbable scaffold. As reported recently, resorbable scaffolds are basically suited for cell-free

cartilage repair approaches. In a rabbit model, the implantation of fibronectin-coated scaffolds based on HA, polylactic acid (PLLA), and the co-polymer poly (lactic-co-glycolic acid) (PLGA) in osteochondral defects resulted in the formation of bony and cartilaginous repair tissue. However, scaffolds that enhance the migration of reparative cells into the empty scaffold or defect were considered to be advantageous for the treatment of osteochondral defects.[39] In addition to polymer-based scaffolds, cell-free cartilage repair has been shown for various resorbable and nonresorbable biomaterials. For instance, in an ovine model, implantation of a non-resorbable device made of filamentous polyethylene terephthelate enhanced the amount of repair tissue after microfracture treatment compared to microfracture treatment alone.[40] The use of a type II collagen membrane for covering of cartilage defects in dogs pretreated with microfracture showed the best filling with repair tissue, even in comparison to defects treated with autologous chondrocytes embedded in the collagen matrix.[41] Interestingly, in the ovine model, the use of a porcine collagen matrix for covering of cartilage defects after pretreatment with microfracture showed no improvement of defect healing compared to microfracture treatment. However, a hyaline-like cartilage repair tissue formed after microfracture and covering of the defects with the collagen matrix augmented with chondrocytes.[42,43]

Obviously, covering of cartilage defects after microfracture is advantageous for the healing sequence, and may enrich the amount of progenitor cells with chondrogenic differentiation potential at the defect site and may enhance the formation of cartilaginous repair tissue. Consequently, covering of cartilage defects pretreated with microfracture with a cell-free collagen matrix combined with fibrin glue and autologous serum is suggested to be a promising treatment option for cartilage defects.[44] In addition, covering of cartilage defects with a matrix may protect the underlying bone and the surrounding cartilage by establishing a transition zone from the defect to the healthy cartilage that may absorb the loads affecting the defect. Textile matrix structures allow the in-growth of cells and keep the blood released by microfracture within the defect. This may open the opportunity to treat defects with, at least, a partly missing cartilage rim. However, clinical studies are needed that show the clinical benefit of covering microfractured cartilage defects.

In summary, we have shown that covering of full-thickness ovine cartilage defects pretreated with microfracture with a cell-free implant of a textile polyglycolic acid scaffold, hyaluronan and autologous serum improved the formation of cartilaginous repair tissue in microfracture. In addition, the textile structure of polymer-based scaffolds allows secure fixation of the implant in the defect by cartilage suturing, trans-osseous suturing, or by resorbable pins.[45–47] Therefore, the implantation of polymer-based cell-free implants into cartilage defects is suggested to be a promising approach for the regeneration of articular cartilage defects after microfracture.

ACKNOWLEDGMENTS

The authors are very grateful to Samuel Vetterlein for the excellent technical assistance. This study was supported by grants from the Investitionsbank Berlin and the European Regional Development Fund (grant 10023712, 10129206).

REFERENCES

1. Steadman JR, Rodkey WG, Rodrigo JJ. 2001. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res 391:S362–S369.
2. Matsusue Y, Yamamuro T, Hama H. 1993. Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 9:318–321.
3. Hubbard MJ. 1996. Articular debridement versus washout for degeneration of the medial femoral condyle. A five-year study. J Bone Joint Surg [Br] 78:217–219.
4. Brittberg M, Lindahl A, Nilsson A, et al. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895.
5. Browne JE, Anderson AF, Arciero R, et al. 2005. Clinical outcome of autologous chondrocyte implantation at 5 years in US subjects. Clin Orthop Relat Res 436:237–245.
6. Knutsen G, Engebretsen L, Ludvigsen TC, et al. 2004. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg [Am] 86-A:455–464.
7. Peterson L, Minas T, Brittberg M, et al. 2000. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 374:212–234.
8. Magnussen RA, Dunn WR, Carey JL, et al. 2008. Treatment of focal articular cartilage defects in the knee: a systematic review. Clin Orthop Relat Res 466:952–962.
9. Nehrer S, Domayer S, Dorotka R, et al. 2006. Three-year clinical outcome after chondrocyte transplantation using a hyaluronan matrix for cartilage repair. Eur J Radiol 57:3–8.
10. Bartlett W, Skinner JA, Gooding CR, et al. 2005. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg [Br] 87:640–645.
11. Ossendorf C, Kaps C, Kreuz PC, et al. 2007. Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results. Arthritis Res Ther 9:R41.
12. Pridie KH. 1959. A method of resurfacing osteoarthritic knee joints. J Bone Joint Surg [Br] 41:418–419.
13. Johnson LL. 2001. Arthroscopic abrasion arthroplasty: a review. Clin Orthop Relat Res 391:S306–S317.
14. Endres M, Neumann K, Haupl T, et al. 2007. Synovial fluid recruits human mesenchymal progenitors from subchondral spongious bone marrow. J Orthop Res 25:1299–1307.
15. Ponte AL, Marais E, Gallay N, et al. 2007. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells 25:1737–1745.
16. Ringe J, Strassburg S, Neumann K, et al. 2007. Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. J Cell Biochem 101:135–146.
17. Neumann K, Dehne T, Endres M, et al. 2008. Chondrogenic differentiation capacity of human mesenchymal progenitor cells derived from subchondral cortico-spongious bone. J Orthop Res 26:1449–1456.

18. Steadman JR, Rodkey WG, Briggs KK, et al. 1999. The microfracture technic in the management of complete cartilage defects in the knee joint. Orthopade 28:26–32.
19. Shapiro F, Koide S, Glimcher MJ. 1993. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg [Am] 75:532–553.
20. Knutsen G, Drogset JO, Engebretsen L, et al. 2007. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg [Am] 89:2105–2112.
21. Steadman JR, Briggs KK, Rodrigo JJ, et al. 2003. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 19:477–484.
22. Kreuz PC, Steinwachs MR, Erggelet C, et al. 2006. Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis Cartilage 14:1119–1125.
23. Frisbie DD, Oxford JT, Southwood L, et al. 2003. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop Relat Res 407:215–227.
24. Erggelet C, Neumann K, Endres M, et al. 2007. Regeneration of ovine articular cartilage defects by cell-free polymer-based implants. Biomaterials 28:5570–5580.
25. Erggelet C, Sittinger M, Lahm A. 2003. The arthroscopic implantation of autologous chondrocytes for the treatment of full-thickness cartilage defects of the knee joint. Arthroscopy 19:108–110.
26. O'Driscoll SW, Keeley FW, Salter RB. 1986. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg [Am] 68:1017–1035.
27. Pineda S, Pollack A, Stevenson S, et al. 1992. A semiquantitative scale for histologic grading of articular cartilage repair. Acta Anat (Basel) 143:335–340.
28. Wakitani S, Goto T, Pineda SJ, et al. 1994. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg [Am] 76:579–592.
29. Gudas R, Kalesinskas RJ, Kimtys V, et al. 2005. A prospective randomized clinical study of mosaic osteochondral autologous transplantation versus microfracture for the treatment of osteochondral defects in the knee joint in young athletes. Arthroscopy 21:1066–1075.
30. Buckwalter JA, Brown TD. 2004. Joint injury, repair, and remodeling: roles in post-traumatic osteoarthritis. Clin Orthop Relat Res 423:7–16.
31. Fiedler J, Roderer G, Gunther KP, et al. 2002. BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. J Cell Biochem 87:305–312.
32. Hoemann CD, Sun J, McKee MD, et al. 2007. Chitosan-glycerol phosphate/blood implants elicit hyaline cartilage repair integrated with porous subchondral bone in microdrilled rabbit defects. Osteoarthritis Cartilage 15:78–89.
33. Sekiya I, Larson BL, Vuoristo JT, et al. 2005. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. Cell Tissue Res 320:269–276.
34. Barry F, Boynton RE, Liu B, et al. 2001. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Exp Cell Res 268:189–200.
35. Johnstone B, Hering TM, Caplan AI, et al. 1998. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238:265–272.
36. Hegewald AA, Ringe J, Bartel J, et al. 2004. Hyaluronic acid and autologous synovial fluid induce chondrogenic differentiation of equine mesenchymal stem cells: a preliminary study. Tissue Cell 36:431–438.
37. Kang SW, Bada LP, Kang CS, et al. 2008. Articular cartilage regeneration with microfracture and hyaluronic acid. Biotechnol Lett 30:435–439.
38. Lisignoli G, Cristino S, Piacentini A, et al. 2005. Cellular and molecular events during chondrogenesis of human mesenchymal stromal cells grown in a three-dimensional hyaluronan based scaffold. Biomaterials 26:5677–5686.
39. Solchaga LA, Temenoff JS, Gao J, et al. 2005. Repair of osteochondral defects with hyaluronan- and polyester-based scaffolds. Osteoarthritis Cartilage 13:297–309.
40. Seedhom BB, Luo ZJ, Goldsmith AJ, et al. 2007. In-situ engineering of cartilage repair: a pre-clinical in-vivo exploration of a novel system. Proc Inst Mech Eng [H] 221:475–488.
41. Breinan HA, Martin SD, Hsu HP, et al. 2000. Healing of canine articular cartilage defects treated with microfracture, a type-II collagen matrix, or cultured autologous chondrocytes. J Orthop Res 18:781–789.
42. Dorotka R, Bindreiter U, Macfelda K, et al. 2005. Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair. Osteoarthritis Cartilage 13:655–664.
43. Dorotka R, Windberger U, Macfelda K, et al. 2005. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials 26:3617–3629.
44. Behrens P. 2005. Matrix-coupled microfracture—a new concept for cartilage defect repair [in German]. Arthroskopie 18:193–197.
45. Drobnic M, Radosavljevic D, Ravnik D, et al. 2006. Comparison of four techniques for the fixation of a collagen scaffold in the human cadaveric knee. Osteoarthritis Cartilage 14:337–344.
46. Knecht S, Erggelet C, Endres M, et al. 2007. Mechanical testing of fixation techniques for scaffold-based tissue-engineered grafts. J Biomed Mater Res B Appl Biomater 83B:50–57.
47. Petersen W, Zelle S, Zantop T. 2008. Arthroscopic implantation of a three dimensional scaffold for autologous chondrocyte transplantation. Arch Orthop Trauma Surg 128:505–508.