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A Cell-Free Collagen Type I Device for the Treatment of Focal Cartilage Defects

*Karsten Gavenis, †Bernhard Schmidt-Rohlfing, *Stefan Andereya, *Torsten Mumme, ‡Ulrich Schneider, and *Ralf Mueller-Rath
Departments of *Orthopaedic Surgery; †Trauma Surgery, Aachen University Hospital, Aachen; and ‡Arthro Nova Clinic, Tegernsee, Germany

Abstract: The purpose of this study was to evaluate the potential value of a cell-free collagen type I gel plug for the treatment of focal cartilage defects. Cellular migration and proliferation was addressed in vitro, and the formation of repair tissue in a nude mouse-based defect model. A cell-free plug made of collagen type I was placed in the center of an incubation plate. Surrounding space was filled with a collagen type I gel (Arthro Kinetics, Esslingen, Germany) seeded with 2×10^5 human articular chondrocytes/mL gel. After cultivation for up to 6 weeks in vitro, samples were subject to histological and immunohistochemical staining and gene expression analysis. Subsequently, chondral defects of human osteochondral blocks were treated with the plug, and specimens were cultivated subcutaneously in nude mice for 6 weeks. The repair tissue was evaluated macroscopically, and collagen type II production was investigated immunohistochemically. In vitro, morphology of immigrated cells did not show any differences, as did collagen type II gene expression. After 4 weeks, the plug was homogeneously inhabited. After 6 weeks of cultivation in nude mice, collagen gel plug treatment led to a macroscopically excellent repair tissue. Histological staining revealed a tight bonding, and the collagen gel plug started to be remodeled. We conclude that the novel collagen gel plug device offers an environment favorable for the migration of articular chondrocytes and leads to a good-quality repair tissue in the nude mouse model. The arthroscopic transplantation of a collagen gel plug may be one option in the treatment of focal cartilage defects. **Key Words:** Cartilage repair—Human articular chondrocyte—Collagen gel plug—Tissue engineering.

INTRODUCTION

Tissue engineering has become an important part within the treatment concepts for articular cartilage defects of human knee joints. Among the growing range of carrier systems for matrix-coupled chondrocyte transplantation, collagen gels have gained special attraction. They have proven to promote cellular proliferation while preserving the chondrocyte phenotype (1). Moreover, it is possible to achieve a homogeneous cellular distribution.

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Address correspondence and reprint requests to Dr. Karsten Gavenis, Department of Orthopedic Surgery, Aachen University Hospital, Pauwelsstr. 30, 52074 Aachen, Germany. E-mail: kgavenis@ukaachen.de

When cartilage defects are treated by transplantation of autologous chondrocytes, this has to be done in a two-step procedure. In a first step, chondrocytes are enzymatically released from cartilage biopsies and are either cultivated until a larger cell number is obtained (autologous chondrocyte transplantation, matrix-coupled autologous chondrocyte transplantation [MACT/MACI] and others) (2,3), or directly brought into the carrier (CaReS [Arthro Kinetics, Esslingen, Germany] and others) (4). In a second step, the cell suspension or chondrocyte-seeded carrier is transplanted. These operative techniques are time and money consuming, therefore concentrating on the therapy of larger cartilage defects.

Today, smaller cartilage defects of about 1 cm² can be treated arthroscopically without the application of sophisticated tissue engineering techniques. Recent techniques for the arthroscopic treatment of focal cartilage lesions include microfracturing (5) and mosaicplasty (6). Although the short-term outcome is good, these techniques mainly induce the creation of fibrocartilage, which potentially might lead to degenerative joint diseases (7).

To overcome these limitations, we developed a novel device for the therapy of focal cartilage defects. This noncell-based device consists of a collagen gel plug of standardized size and potentially can be applied arthroscopically. Our hypothesis was that this gel should trigger chondrocyte ingrowth, leading to cartilage repair. Chondrocyte migration has been recently reported in vitro (isolated cell systems) and ex vivo (cartilage organ cultures) (8). The device is based on a rat tail collagen type I gel already in clinical application for matrix-coupled chondrocyte transplantation (CaReS). The purpose of the study was to evaluate the potential value of the collagen plug for focal cartilage repair, focusing on chondrocyte migration into the plug, biochemical characterization of the ingrowing chondrocytes, and the histological evaluation of in vivo generated repair tissue (common nude mouse model).

MATERIALS AND METHODS

The collagen plug device

Collagen type I plugs were obtained from Arthro Kinetics. They consisted of 4,8 mg/mL rat tail collagen type I gel, a gel already in clinical application (CaReS, Arthro Kinetics). The diameter of the samples was 9 mm, the height 3 mm. The plugs were supplied in phosphate-buffered saline solution and stored at 4°C until needed.

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Cell migration assay

To study cell migration into the plug, human articular cartilage was obtained from the femoral condyles of 10 patients (2 male, 8 female; mean age 68.7 years) suffering from severe osteoarthritis and undergoing total knee replacement. Written consent was obtained from the patients prior to operation. Articular chondrocytes were released by enzymatic digestion, and a rat tail collagen type 1 gel (Arthro Kinetics) was seeded with 2×10^5 chondrocytes/mL gel. The collagen concentration of this gel was 6 mg/mL.

For each time point, a cell-free collagen gel plug was placed in the center of a 12-well incubation plate, and the surrounding space was filled with 1.5-mL cell-seeded collagen gel. After gelling, samples were overlaid with Dulbecco's modified Eagle's medium supplemented with 10% human serum, 100 U/mL penicillin, and 100 µg/mL streptomycin, and cultivated for up to 6 weeks. For each patient, this set-up was performed twice, one specimen serving for histological examination while the other was prepared for gene expression analysis.

Human condylus defect model

Human osteochondral blocks were obtained from 10 patients (3 male, 7 female, mean age 73.5) undergoing total knee replacement. All patients gave their written consent prior to operation. Subsequently, freshly prepared specimens were trimmed, and chondral defects of 8-mm diameter were drilled. The defects were filled press-fit under sterile conditions with the collagen gel plug and cultivated subcutaneously in nude mice for 6 weeks. Nude mouse anesthesia was performed by isofuran treatment. After performing a 2-cm skin cut, the osteochondral block could be placed on the back of the mouse. After recovery, samples were subject to standard hematoxylin/eosin (HE) staining and collagen type II immunostaining.

Histological and immunohistochemical staining

HE staining was performed according to standard protocols. To obtain immunostainings, slices of the samples were deparaffinized, blocked for 1 h with 1% normal goat serum and incubated with a polyclonal antibody to human collagen type II (Biotrend, Cologne, Germany) overnight. Staining was visualized using the streptavidin/biotin technique (Vectastain ABC Kit, Vector Laboratories, Burlingame, VT, USA). Discrimination of human and mouse-derived cells after subcutaneous cultivation in nude mice was performed by overnight incubation with culture supernatant of a monoclonal antibody raised against human chondrocytes (clone no. 71.4, kindly provided by the Institute of Immunology, University of Heidelberg, Germany). To rule out a potential cross-reactivity, this clone was tested on different mouse tissues.

Analysis of mRNA expression

To quantify gene expression of collagen type II and type I, collagen gel plug and surrounding collagen gel were separated. RNA was isolated with the Oligotex Direct mRNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Isolated mRNA was transcribed into cDNA by the SuperScript II First Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Gene expression was quantified by real-time polymerase chain reaction (PCR) on a LightCycler (Roche Diagnostics, Indianapolis, MN, USA). Β-actin was used as an internal standard. Primer sequences and PCR program have been described in detail elsewhere (4).

Quantitative PCR was evaluated by an ANOVA test, with repeated measurements using the SPSS statistical software (version 12.0, SPSS, Inc., Chicago, IL, USA).

RESULTS

After 1 week, human articular chondrocytes started to migrate from the cell-seeded, surrounding collagen gel into the cell-free collagen plug core (Fig. 1A). After 6 weeks of in vitro cultivation, the collagen plug was homogeneously inhabited by the chondrocytes. Chondrocytes cultivated in surrounding collagen gel and collagen plug showed no morphological differences. They displayed an intermediate phenotype, revealing some signs of morphological dedifferentiation, namely a fibroblast-like cell-shape. Collagen type II protein was produced to nearly equivalent amounts and stored in the pericellular region of the cells (Fig. 1A).

The number of chondrocytes inhabiting the collagen plug core was increasing from 2.7×10^4 after 1 week of cultivation to 4.7×10^4 after 6 weeks (Fig. 1B). Cell number as determined by CASY cell counter consisted of chondrocytes constantly migrating into the plug and chondrocytes resulting from cellular proliferation. The total cell number of collagen plug and surrounding collagen gel was increasing from 1.77×10^5 after 1 week to 2.66×10^5 after 6 weeks.

Collagen type I and type II gene expression revealed no differences between chondrocytes grown in surrounding collagen gel or central plug (data not shown). Generally, collagen type II gene

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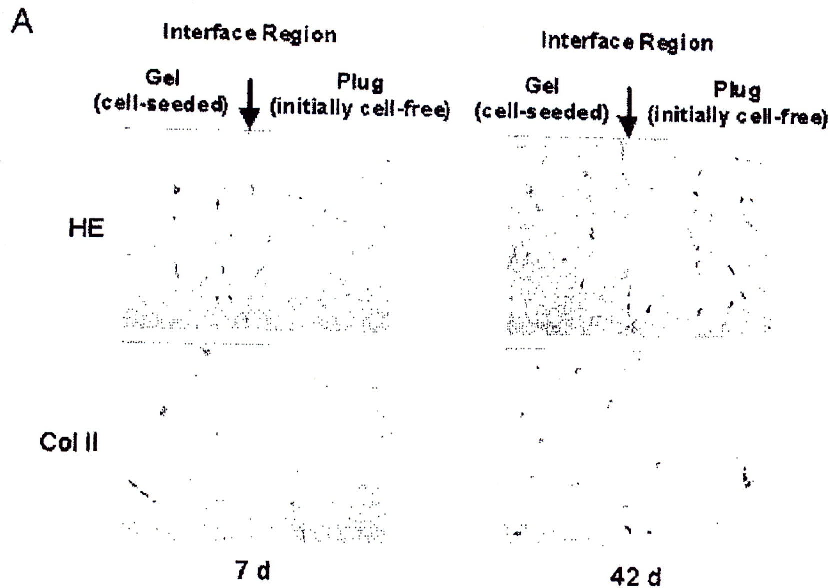

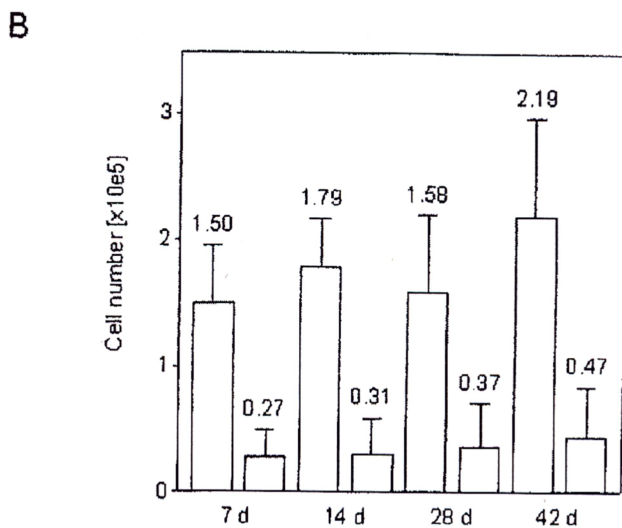

FIG. 1. (A) Hematoxylin/eosin (HE) and collagen type II (Col II) staining of an initially cell-free collagen gel plug embedded in cell-seeded collagen gel and cultivated for 7 days and 42 days in vitro. Shown is the bonding region between initially cell-free plug and cell-seeded collagen gel. Original magnification ×200. (B) Number of chondrocytes in the central collagen gel plug (dotted bars) and in the surrounding collagen gel (open bars). Cell number was determined by CASY cell counter. $n = 10$.

expression was decreasing during in vitro cultivation, while collagen type I gene expression constantly increased.

Subsequently, chondral defects of human osteochondral blocks were press-fit filled with the collagen plug under sterile conditions (Fig. 2A). After 6 weeks of in vivo cultivation subcutaneously in nude mice, the specimens showed a smooth surface of the repair tissue (Fig. 2A). Histological examinations revealed a tight bonding to the surrounding cartilage (Fig. 2B). Cells started to migrate from the surrounding cartilage into the plug, although, after 6 weeks, no homogeneous cell distribution has been obtained. A monoclonal antibody used to discriminate mouse and human cells revealed that cells inhabiting the plug were both of human and mouse origin (Fig. 2C). This antibody detected human chondrocytes immigrating a collagen gel in vitro and cells of human condyles after in vivo cultivation, but was negative on different mouse tissues. Moreover, cellular distribution suggests that chondrocytes from the surrounding cartilage immigrated the deeper layers of the plug. Again, collagen type II protein was stored mainly pericellularly, and the repair tissue displayed a weaker staining than the surrounding cartilage. At this experimental stage, no scaling was performed to quantify the repair tissue quality.

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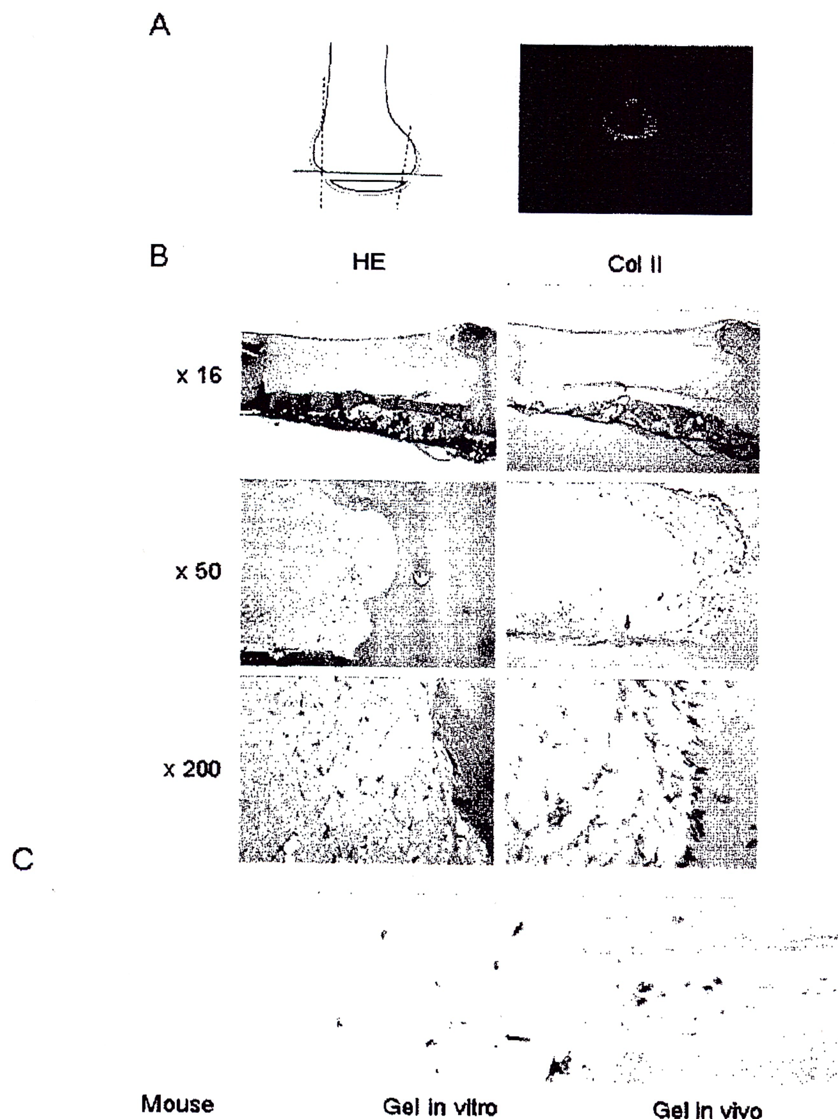

FIG. 2. (A) Schematic view of human osteochondral blocks harvested from patients undergoing total knee replacement. Chondral defects were filled with initially cell-free collagen gel plug; specimens are shown after 6 weeks of subcutaneous cultivation in nude mouse. (B) Hematoxylin/eosin (HE) staining and collagen type II (Col II) immunostaining of osteochondral blocks after 6 weeks of subcutaneous cultivation in nude mouse. The bonding region reveals a high cell number and a tight bonding. Original magnification ×16, 50, and 200. (C) Immunostaining of a monoclonal antibody raised against human chondrocytes on mouse kidney (left), human chondrocytes immigrating a collagen gel in vitro (middle) and human osteochondral blocks after 6 weeks of subcutaneous cultivation in nude mouse. Original magnification ×630.

DISCUSSION

Tissue engineering techniques are increasingly used to treat symptomatic knee defects. To date, no clear guidelines exist for the use and indications of these techniques, but in general, they are applied to larger defects of about 3 cm^2. Because of their advantages for the cultivation of chondrocytes, collagen gels have gained attraction as a three-dimensional culture system. They have demonstrated their capacity to promote chondrocyte proliferation and proteoglycan synthesis in vitro (9,10).

When intended for clinical application, matrix materials have to be chosen with special care. In the presented study, we used a collagen gel made of tails of inbreeding rats raised for collagen supply exclusively. This gel has been used for the treatment of articular cartilage defects for some years (CaReS, Arthro Kinetics) (4,11,12). Additionally, collagen gel plugs could potentially be produced in any required size. The in vitro cell migration assay performed in this study revealed no differences between chondrocytes initially seeded in the collagen gel or immigrating the plug.

Although it is still under debate if small cartilage lesions should be treated at all, for several reasons, the development of tissue-engineering methods for the arthroscoptic treatment of focal cartilage defects is intensified. A tissue-engineered plug can be adapted to the actual defect size in order to achieve an optimal fit. The implantation of a tissue-engineered device may be performed arthro-

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scopically, minimizing surgical time and effort. Furthermore, there is no need to harvest autologous osteochondral samples, therefore eliminating donor site morbidity.

Some efforts have been made to develop a matrix construct for the treatment of smaller cartilage defects. Schaefer et al. developed a cartilage/bone composite and cultivated it in vitro, but never tested the reparative capacity in vivo (13), while Ossendorf et al. implanted a cell-seeded, two-component gel-polymer scaffold (BioSeed-C, BioTissue, Freiburg, Germany) arthrotomically and arthroscopically into posttraumatic or degenerative cartilage lesions. Two-year follow-up of 40 patients revealed a significant improvement with regard to the modified Cincinnati and Lysholm score, as well as the formation of a cartilaginous repair tissue (14). Additional efforts have been made by Liu et al. (15) and Ito et al., who produced chondral plugs designed for the treatment of osteochondral cartilage defects by cultivating rabbit autologous chondrocytes on an atelocollagen (a bovine collagen type I gel)/PLLA mesh composite (16). When we treated chondral defects in human osteochondral blocks with our cell-free collagen plug and cultivated them for 6 weeks in nude mice, repair tissue displayed a smooth surface and a tight bonding. Moreover, the outer parts of the repair tissue were densely inhabited by immigrating chondrocytes and some mouse-derived cells, although cellular migration was slower than from cell-seeded gel in vitro. This may be due to the tighter structure of the extracellular matrix in articular cartilage compared with an artificial collagen gel. Migration of articular chondrocytes in vitro and in vivo has been reported, recently reviewed by Morales (8). Although the nude mouse is a well-established system for the cultivation of collagen gel specimens, no articular setting is provided, and, especially, mechanical loading is missing. Therefore, the next step will be to validate the results treating cartilage defects in large animals where an appropriate mechanical loading is provided.

CONCLUSION

In this study, we present a collagen gel plug intended for the treatment of focal cartilage lesions. It has proven to promote chondrocyte in-growth and proliferation in vitro. We believe that this cell-free plug has the potential to become a device for the arthroscopic treatment of focal cartilage defects.

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