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Autologous Collagen-Induced Chondrogenesis Using Fibrin and Atelocollagen Mixture

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Key Words
Atelocollagen · Cartilage · Knee · Fibrin · Rabbit

Abstract
For articular cartilage defect treatment, many treatment modalities have been developed. We evaluate the cartilage repair potential of an atelocollagen and fibrin mixture transplanted to cartilage defects. A circular, articular cartilage defect 4 mm in diameter was made in the trochlear region in each of 20 New Zealand white rabbits. The 10 rabbits in the control group were kept without treatment and the 10 rabbits in the experimental group underwent injection of atelocollagen mixed with fibrin. At week 12 following surgery the cartilage was observed and histologically compared in both groups. The surface of the newly generated cartilage was very smooth and even, and we also noted that the entire area was completely regenerated in the experimental group. The control group showed incomplete and irregular cartilage formation in the defect. Regarding the histological scoring, comparison of the two groups differed significantly ($p < 0.001$). Injection of a mixture of atelocollagen and fibrin used to treat articular cartilage defects of the knee appears to be an effective method for cartilage regeneration.

Introduction

Since articular cartilage is avascular, superficial injury does not induce as sufficient an inflammatory reaction as that which would lead to repair in other types of tissue. Therefore, the regenerative capacity of cartilage is seen to be limited and injury can lead to degeneration of the fibrillar collagen structure [Buckwalter, 1998]. Methods used to treat damaged articular cartilage include arthroscopic debridement, microfracture, drilling, osteochondral transplantation and autologous chondrocyte implantation (ACI). Of these methods, ACI has become established as the most successful method for attempting to recover normal articular cartilage. To date, however, ACI requires two surgeries in which part of the normal articular cartilage is extracted and then transplanted fol-

Abbreviations used in this paper

ACI	autologous chondrocyte implantation
GAG	glycosaminoglycan
SEM	scanning electron microscopy
TEM	transmission electron microscopy

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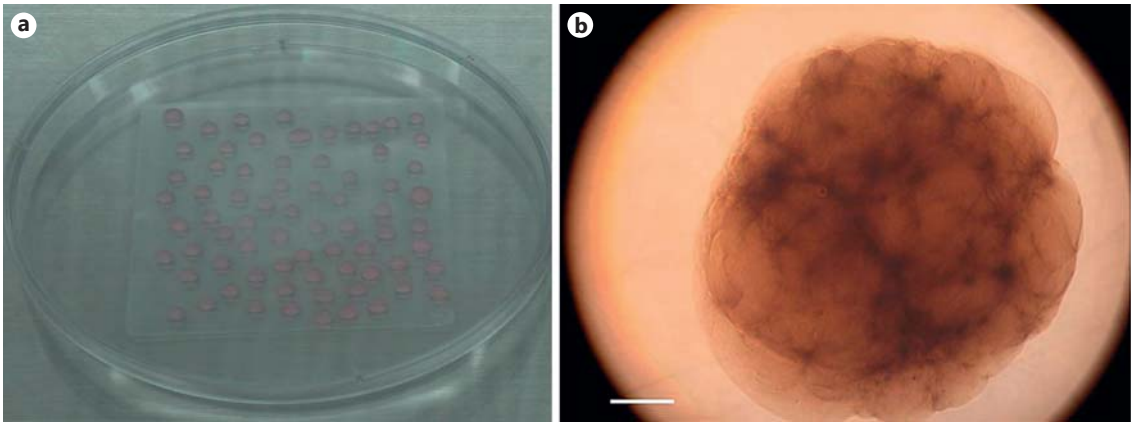

Fig. 1. Stable hydrogel collagen bead formation with 4 mg/ml atelocollagen solution (**a**) and MSC-collagen gel bead at 48-hour culture (**b**). Scale bar = 0.5 mm.

lowing culturing, and this method is expensive [Lubowitz et al., 2007]. In this regard, the treatment methods for chondromalacia or small-sized cartilaginous lesions using previous surgical methods have demonstrated the increasing necessity for developing a simplified technique.

Microfracture continues to be used despite the regenerated cartilage being mechanically weaker fibrocartilage, rather than hyaline cartilage, because the short-term results are successful and it is a simple, cost-effective technique [Williams and Harnly, 2007]. Efforts are currently being developed to improve the outcomes by facilitating the regeneration of articular cartilage after the microfracture procedure using a bio-scaffold [Benthien and Behrens, 2011; Piontek et al., 2012].

Following the microfracture, while using a mixture of atelocollagen and fibrin as a scaffold, attempts have been made to examine the possibilities for the treatment of cartilaginous defects. In vitro evaluation of MSCs embedded in 3D atelocollagen was also performed with respect to their ability to survive and differentiate into chondrogenic cells in this scaffold.

Materials and Methods

Cell Survival and Chondrogenic Differentiation of Mesenchymal Stem Cells in the Fibrin and Atelocollagen Mixture

Culture of Human Mesenchymal Stem Cells

Human mesenchymal stem cells in passage 2 and derived from bone marrow (Promocell, Heidelberg, Germany) were cultured for two more passages using MSC growth medium (Promocell) and according to the manufacturer's instructions. A proliferation medium consisting of Dulbecco's modified Eagle's medium-low glucose (PAA, Pasching, Austria), 20% fetal bovine serum (PAA), 100 U/ml penicillin and 100 μg/ml streptomycin, was used for further passages, unless otherwise specified. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. After washing with Hank's Balanced Salt Solution without calcium or magnesium (PAA), 0.25% Trypsin/1 mM EDTA (Gibco-Invitrogen, Carlsbad, Calif., USA) was used to detach cells for subculture. Human mesenchymal stem cells expanded up to the 4th passages were used for further experiments.

Encapsulation of MSCs in Collagen Gel Beads

Mesenchymal stem cells at a final concentration of 2.5×10^5 cells/ml were mixed with neutralized porcine type I atelocollagen solution (UBIOSIS, Seongnam, South Korea) at a final concentration of 4 mg/ml. The mixture was then dropped onto sterile parafilm on a Petri dish (fig. 1a). The drops were incubated for 45 min at 37°C in order to induce collagen gelling (fig. 1b). The beads produced were then collected by adding warmed culture media to detach them from the parafilm. The beads were distributed in four, 50-ml, conical centrifuge tubes (BD-Falcon; BD Biosciences, San Jose, Calif., USA). The volume of each drop was 60 μl and 15,000 cells were included in each drop. About 40 drops were added to each 50-ml tube.

Proliferation medium was added to each tube and was incubated for 2 days in a 5% CO_2 incubator (Forma Scientific, Marietta, Ohio, USA) at 37°C in order to allow the cells to adapt to the bead culture environment. Beads in the four tubes were allocated to two groups, i.e. the control group and the chondrogenic differentiation group. The control beads were cultured in proliferation medium, while the chondrogenic differentiation beads were cultured in chondrogenic differentiation medium during the differentiation culture period. The chondrogenic differentiation medium consisted of Dulbecco's modified Eagle's medium-high glucose (PAA) containing 10 ng/ml transforming growth factor-β3, 25 μg/ml ascorbic acid, 25 μM ascorbic acid-2-phosphate, 100 U/ml penicillin, 100 μg/ml streptomycin and 1% (v/v) ITS plus (all from Sigma-

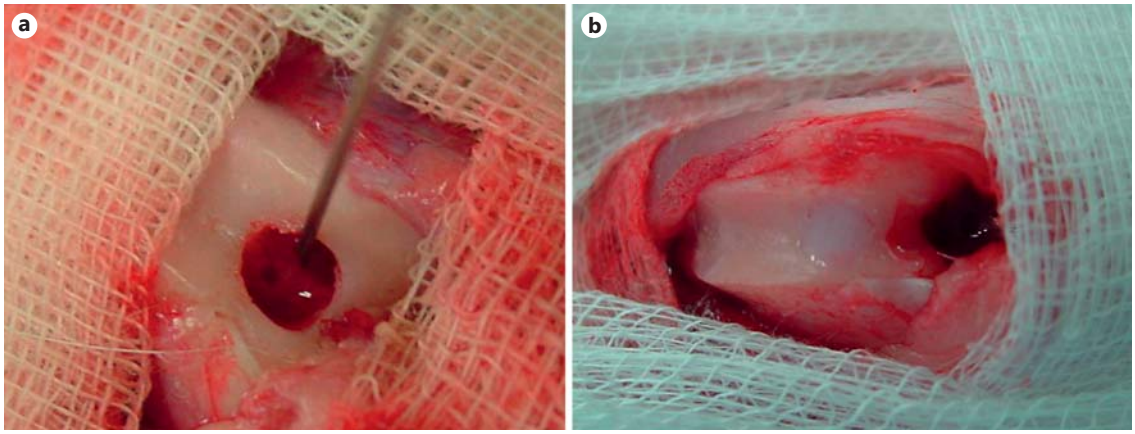

Fig. 2. a A round hole with a diameter of 4 mm down to the subchondral bone was made in the trochlear region and microfracture was performed using a 23-gauge needle. **b** A mixture of atelocollagen and fibrin was injected into the microfractured defect.

Aldrich, St. Louis, Mo., USA). The media were changed twice weekly for 3 weeks.

Analysis of Chondrogenic Phenotype Differentiation

MSC beads were washed twice with Hank's Balanced Salt Solution after removal of the culture medium by suction. The beads were then incubated at 37°C in a 5% CO_2-humidified atmosphere.

Either Alcian blue (10 mg/ml; Sigma-Aldrich) solution in 3% acetic acid or 10 mg/ml safranin O (Sigma-Aldrich) in distilled water was used to detect glycosaminoglycan (GAG) in the samples. Alcian blue solution was applied for 12 h at room temperature after washing the fixed beads with distilled water and 3% acetic acid. Safranin O solution was applied for 5 min at room temperature after the fixed beads were washed with distilled water.

Microstructures in the MSC-Collagen Gel Beads

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to analyze the microstructural morphologies in MSC-collagen gel beads cultured under the chondrogenic condition. Briefly, beads were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer overnight, then washed in 0.1 M phosphate buffer and postfixed with 1% osmium tetroxide in the same buffer for 1 h. The beads were dehydrated using a graded ethyl alcohol series and acetone, and then embedded in Epon 812 (epoxy resin) for TEM processing. Ultrathin sections (70~80 nm) were obtained using an ultramicrotome (Leica Ultracut UCT; Leica Microsystems, Wetzlar, Germany), which were then double stained with uranyl acetate/lead citrate and examined using a JEM 1010 unit (JEOL, Tokyo, Japan) at 60 kV. After dehydration, specimens were transferred to hexamethyldisilazane and allowed to air dry for SEM processing. All samples were coated with gold using a sputter coater and were examined using a JSM-5410LV unit (JEOL). The SEM was operated at an accelerating voltage of 15 kV.

Cell Distribution and Viability in the Collagen Gel Beads

Fluorescent dyes were used to distinguish viable cells from dead cells in the slices of incubated MSC-collagen beads. In brief, the beads were cut into 1-mm sections using a blade after 4 days of cultivation in the proliferation medium. The sections were incubated in the proliferation medium containing 2 μM calcein acetoxymethylester (Sigma-Aldrich) and 4 μM ethidium homodimer (Sigma-Aldrich) for 30 min before examination using fluorescence microscopy.

Articular Cartilage Defect Repair Using Fibrin and Atelocollagen Mixture

Experimental Animals

Twenty New Zealand white rabbits, each weighing approximately 4 kg, were maintained 1 week prior to the experiments using the same feed and under the same conditions. Among them, 10 animals were used as the atelocollagen transplant group and the remaining 10 animals were the control animals which remained without treatment.

Approval for the animal experiments conducted in this study was obtained from our Institutional Review Board. The experiment was performed in accordance with the ILAR (Institute of Laboratory Animal Resources) Guide for the Care and Use of Laboratory Animals.

Surgical Procedure

The rabbits were anesthetized with ketamine and were placed in a supine position. A longitudinal, midline skin incision was made and extended 3 cm above the superior pole of the patella to the level of the tibia tubercle. Subcutaneous tissue was divided in the line of the skin incision. A medial skin flap was developed in order to expose the quadriceps tendon, the medial border of the patella, and the medial border of the patellar tendon. A medial, parapatellar, capsular incision was then made, and the patella was dislocated laterally in order to expose the femoral condyle.

Defect Formation

A defect was formed up to the subchondral bone by making a lesion 4 mm in diameter. Then, using a 23-gauge needle, three holes were made in the subchondral bone (fig. 2a).

Injection of Atelocollagen and Fibrin Mixture

For the injection procedure, we used two, 1-ml syringes and a Y-shaped mixing catheter. In one syringe, 1 ml of fibrinogen (Greencross, Yongin, South Korea) was filled, and the other syringe was filled with 0.9 ml of atelocollagen and 0.1 ml of thrombin (50 IU). Atelocollagen mixed with fibrin was then slowly injected into the defect area (fig. 2b). In order not to overflow the margin of cartilage defect, the dependent position of the defect site was maintained for 5 min. Flexion and extension motion of the knee was performed 3–5 times in order to check for any graft failure. The wound was then closed layer by layer.

Examination Methods

Gross Appearance

Twelve weeks postoperatively, 10 rabbits from each group were sacrificed and the areas of cartilage defect as well as portions of the normal tissue were extracted. The gross appearance of the newly generated cartilage was evaluated by comparing it with normal tissue [Breinan et al., 1998; Buckwalter, 1999; O'Driscoll et al., 2001].

Histochemical Staining

Neocartilage and normal cartilage were extracted from each rabbit in each group and were fixed using 3.7% neutral-buffered formalin (Sigma-Aldrich, Poole, UK). The cartilage samples were then immersed and decalcified in the decalcifying solution diluted with a formic acid:nitric acid:H_2O ratio of 25:5:70. After decalcification, the samples were dipped into and neutralized in 5% sodium sulfite solution (Sigma-Aldrich), and then rinsed with water. They were then immersed for 1 h in each of 50, 60, 70, 80, 90 and 95% ethanol solutions as well as in absolute ethanol so as to be gradually dehydrated. After dehydration, they were immersed in xylene (Duksan, Ansan, South Korea) and then infiltrated with paraffin so they could be cut into blocks. Sections were cut from each block using a microtome (Leica Microsystems) in order to perform histochemical staining. The sections were then stained with hematoxylin and eosin so as to be able to observe the tissue morphology, and with toluidine blue and Masson's trichrome staining in order to check the collagen of the cartilage as well as the GAG content.

A total of five factors, i.e. cell morphology, matrix staining, surface regularity, thickness of the neocartilage and integrity of the graft with host, were observed, and each cartilage area in the surgical area was evaluated regarding these five features by assigning it a score ranging from 0 to 14 points and representing a modification of that described by Wakitani et al. [1994] (table 1). First, the cell morphology was graded from 0 (normal cartilage) to 4 points (no cartilaginous tissue). Next, matrix staining or the degree of metachromatic staining with toluidine blue was graded from 0 (for tissue that was normal) to 3 points (no metachromatic staining). Third, the smoothness of the surface of the cartilage was assessed and then divided into four grades with 0 points if more than three-quarters of the surface was smooth and 3 points if less than one-quarter of it was smooth. The thickness of the neocartilage was assessed and then divided into three grades. Zero points were given if the thickness was the same as that of the normal cartilage or more than two-thirds that of the surrounding cartilage, and 2 points were given when the average thickness was less than one-third of that of the surrounding cartilage. Lastly, integration of the donor cartilage with the host adjacent cartilage was graded from 0 when there was no gap between the donor and the host cartilage to 2 points when there was a complete lack of integration.

Table 1. Histologic grading scale for the cartilage defects

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 adjacent host cartilage)	0
Slightly reduced	1
Markedly reduced	2
No metachromatic stain	3
Surface regularity[1]	
Smooth (>3/4)	0
Moderate (>1/2–3/4)	1
Irregular (1/4–1/2)	2
Severely irregular (<1/4)	3
Cartilage thickness[2]	
>2/3	0
1/3–2/3	1
<1/3	2
Integration of donor cartilage with adjacent host cartilage	
Both edges integrated	0
One edge integrated	1
Neither edge integrated	2
Total maximum	14

[1] Total smooth area of the reparative cartilage compared with the entire area of the cartilage defect.

[2] Average thickness of the reparative cartilage compared with that of the surrounding cartilage.

Immunohistochemistry

Immunohistochemistry was performed on the same serial sections used in the histochemical staining. Hybridomas for antibodies used in the immunohistochemistry were IH11 for collagen type I (ATCC, Manassas, Va., USA) and CIIC1 for collagen type II (Sigma-Aldrich). IH11 and CIIC1 were separately cultured in 10% Dulbecco's modified Eagle's medium, and the culture media were centrifugally separated. Dot blot analysis was then performed to determine the concentration of an approximate antibody by titration so as to use it as the primary antibody. A universal antibody of the elite ABC kit (Vector, Burlingame, UK) was used as a secondary antibody to induce a secondary antibody reaction, and the DAB reagent (Vector) was used to generate the color reaction.

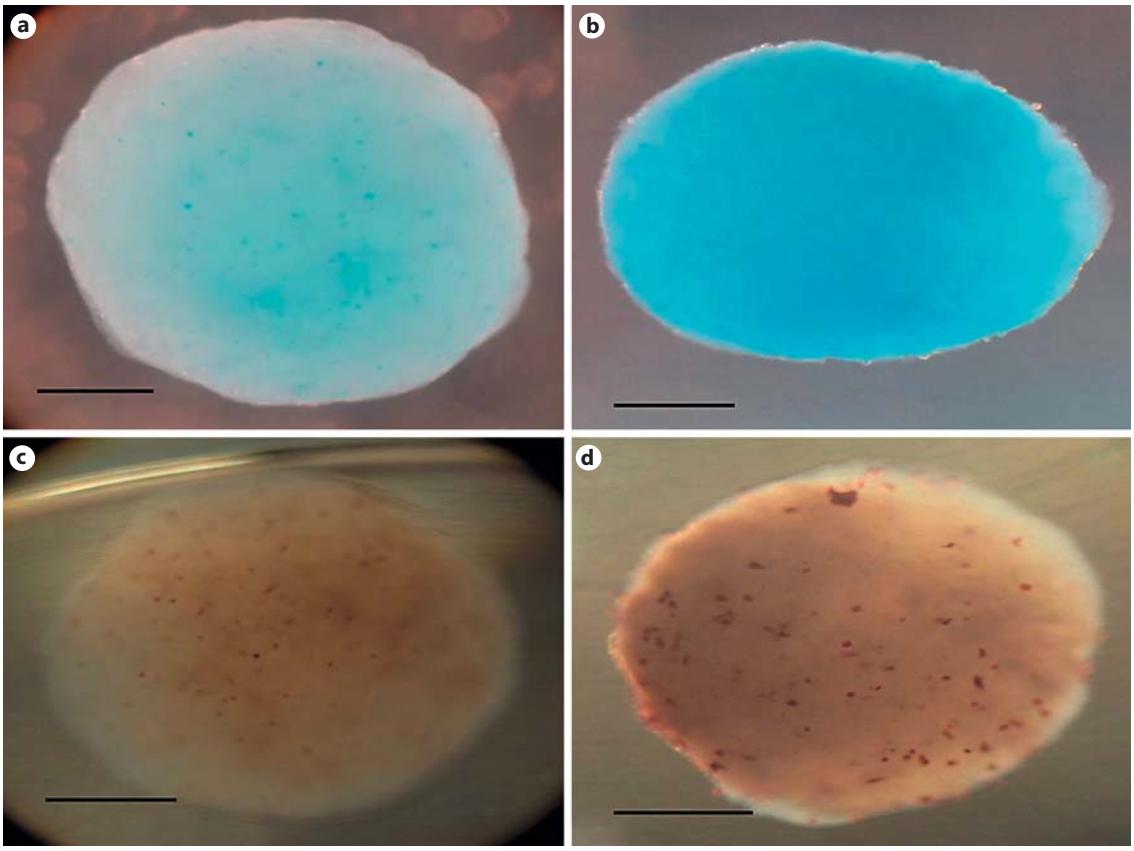

Fig. 3. Alcian blue (**a**, **b**) and safranin O (**c**, **d**) staining of MSC-collagen gel beads under different conditions and performed for GAG detection: control groups (**a**, **c**) and chondrogenic differentiation groups (**b**, **d**). Scale bar = 0.5 mm.

Results

In vitro Analysis of Cell Survival and Chondrogenic Differentiation of Mesenchymal Stem Cells in the Fibrin and Atelocollagen Mixture

Staining for GAG

The control group showed no GAG expression after safranin O staining, whereas the chondrogenic differentiation group showed strong GAG expression. The control group showed mild Alcian blue staining, which was stronger in the chondrogenic differentiation group (fig. 3).

SEM and TEM of Collagen-MSC Hydrogel Beads

SEM pictures revealed the microstructure of MSC-collagen beads which have a coarse surface so as to provide mechanical affinity for cell attachment. SEM and TEM images were obtained in order to distinguish newly biosynthesized collagen from hydrogel collagen and to investigate the more detailed structure within the beads (fig. 4). TEM images revealed newly biosynthesized collagen fibers, and incorporated cells were visible in the TEM pictures of collagen-MSC hydrogel beads (fig. 5).

Cell Distribution and Viability in the Collagen Gel Beads

The initial total cell concentration was adjusted to 250,000 cells per milliliter of MSC-collagen mixture or 4,500 cells per bead during the encapsulation of MSCs in collagen gel beads. The initial viability before gel bead formation was measured to be 96%. Cells in the collagen gel were found to be randomly distributed throughout the gel, based on the microscopic examination of sections after 4 days of incubation of the beads. More than 75% of the cells were found to be viable and showing green fluorescence produced by calcein acetoxymethylester. The re

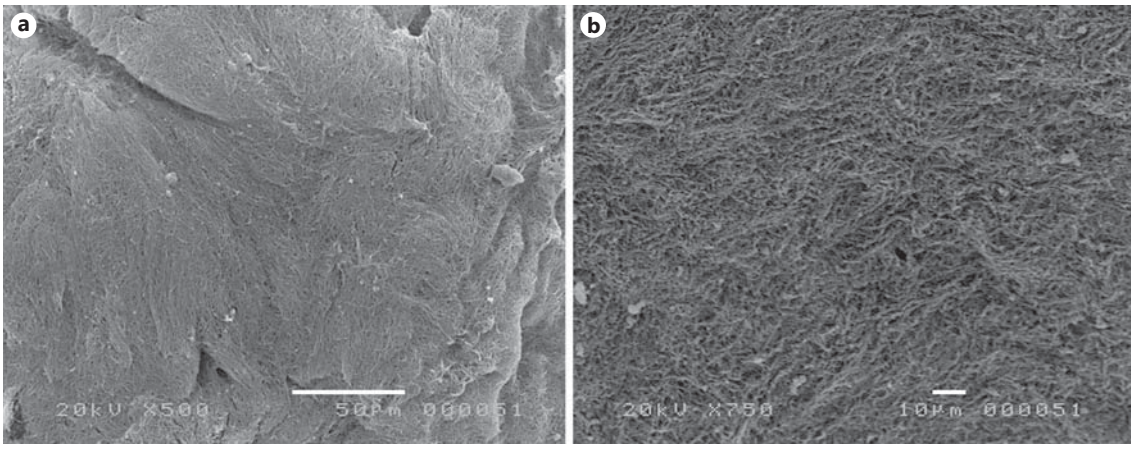

Fig. 4. SEM of the surface (**a**) and the inside (**b**) of MSC-collagen gel beads.

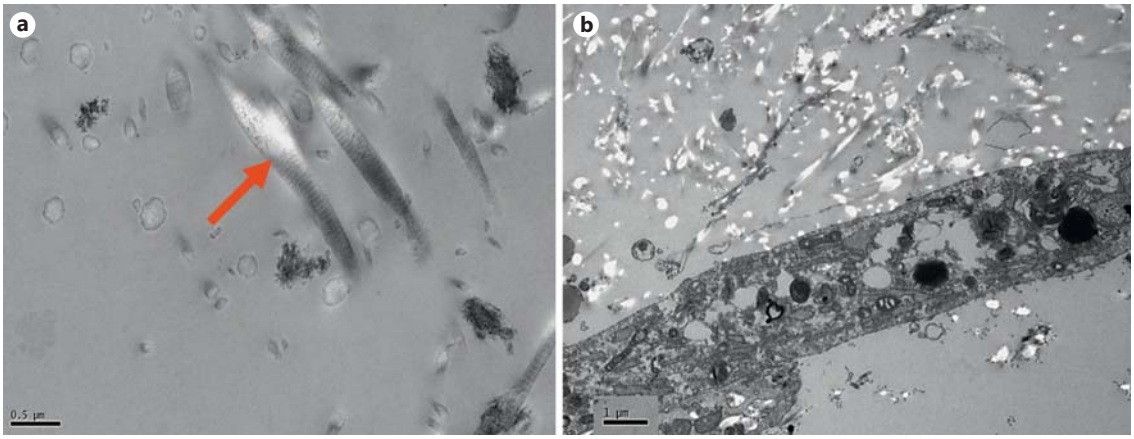

Fig. 5. TEM of MSC-collagen gel beads cultured in a chondrogenic differentiation condition for 21 days. TEM images revealed newly biosynthesized collagen fibers (arrow; **a**), and incorporated cells were visible in the TEM pictures of collagen-MSC hydrogel beads (**b**).

Table 2. Comparison of the histologic findings of the treatment groups

Variables	Experimental (n = 10)	Control (n = 10)	p
Cell morphology	1.300±0.483	2.400±0.516	0.001
Matrix staining (metachromasia)	1.300±0.483	1.800±0.421	0.063
Surface regularity	0.200±0.422	2.000±0.943	0.000
Cartilage thickness	0.100±0.316	1.200±0.789	0.004
Integration of donor cartilage with adjacent host cartilage	0.200±0.422	1.200±0.422	0.001
Total score	3.100±1.853	8.600±2.171	0.000

Values are mean ± SD. To determine p values, Mann-Whitney U test was performed.

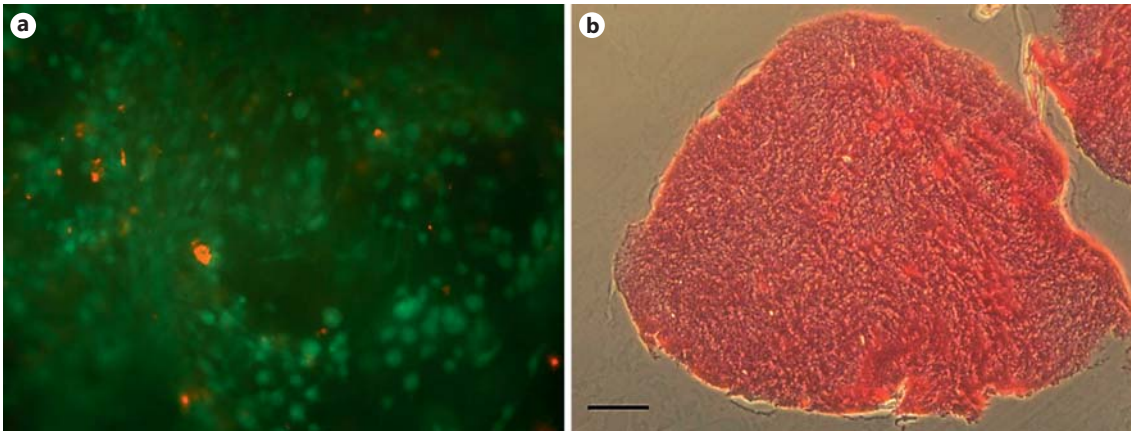

Fig. 6. Sections obtained from MSC-collagen gel beads. **a** Fluorescent dye staining of section taken from 4-day incubated beads. Viable cells appear green and dead cells are red as they were stained with calcein acetoxymethylester and ethidium homodimer, respectively. Original magnification ×40. **b** Sirius red for collagen staining of micro-sectioned beads which had been embedded in a paraffin block after 3 weeks of cultivation. Scale bar = 100 μm.

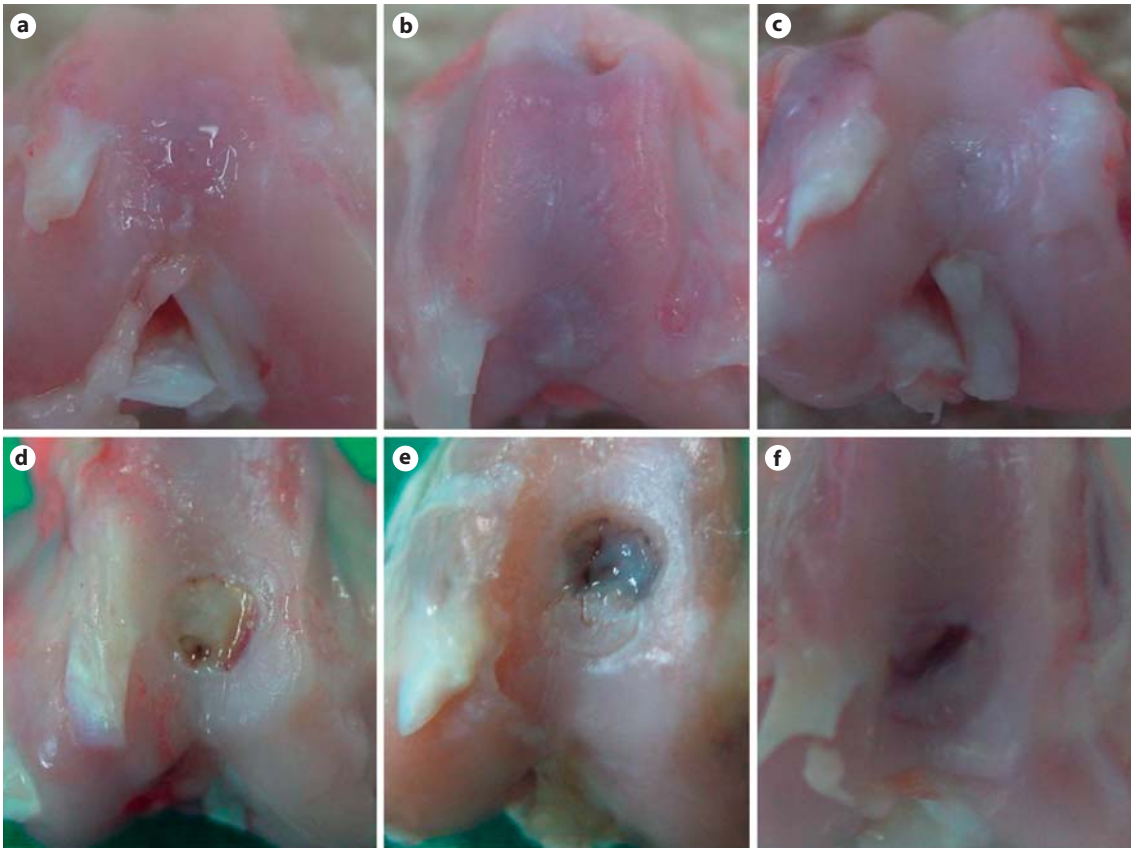

Fig. 7. Gross morphology of the experimental (**a–c**) and control (**d–f**) groups.

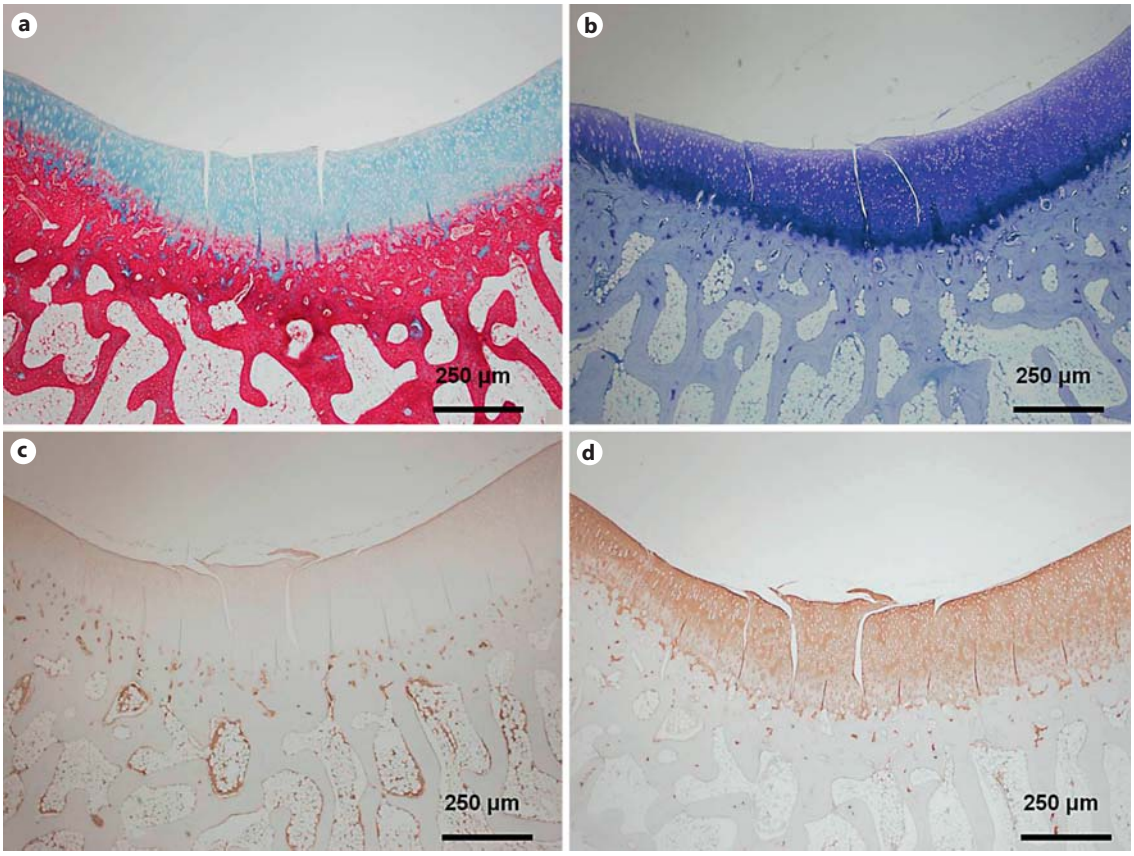

Fig. 8. Photomicrographs of sections of regenerative cartilage with fibrin-atelocollagen mixture 12 weeks after implantation. Masson's trichrome staining (**a**), toluidine blue staining (**b**), immunohistochemical staining with anti-collagen type I antibody (**c**) and immunohistochemical staining with anti-collagen type II antibody (**d**). Original magnification ×40.

maining cells were found to be dead and showing red fluorescence due to ethidium homodimer infiltration into the nucleus (fig. 6).

Articular Cartilage Defect Repair Using a Fibrin and Atelocollagen Mixture

Comparison between the Control Group and the Atelocollagen Treatment Group

Gross Appearance

In both the control and the atelocollagen treatment groups, there was a lack of both tissue constriction and adhesion. There were also no findings suggestive of synovitis. With the atelocollagen treatment group, the cartilage defect was completely filled with neocartilage, the surface was very uniform, and there was a smooth connection with normal tissue. In addition, the cartilage color was even. In the control group, the cartilage defect was not completely filled with neocartilage, the neocartilage color was yellowish and the surface was irregular (fig. 7).

Histological Scoring

Twelve weeks postoperatively, we investigated the surface coverage of the defect and verified the cartilage color and surface smoothness in both groups. We also assessed the thickness of the neocartilage. The two groups were compared using the Mann-Whitney test. Comparison of the two groups showed significant differences ($p < 0.001$; table 2) in that in the atelocollagen treatment group, the coverage, color, surface smoothness and thickness of the neocartilage were nearly complete, whereas in the control group all specimens showed incomplete regeneration and some defects in every evaluation.

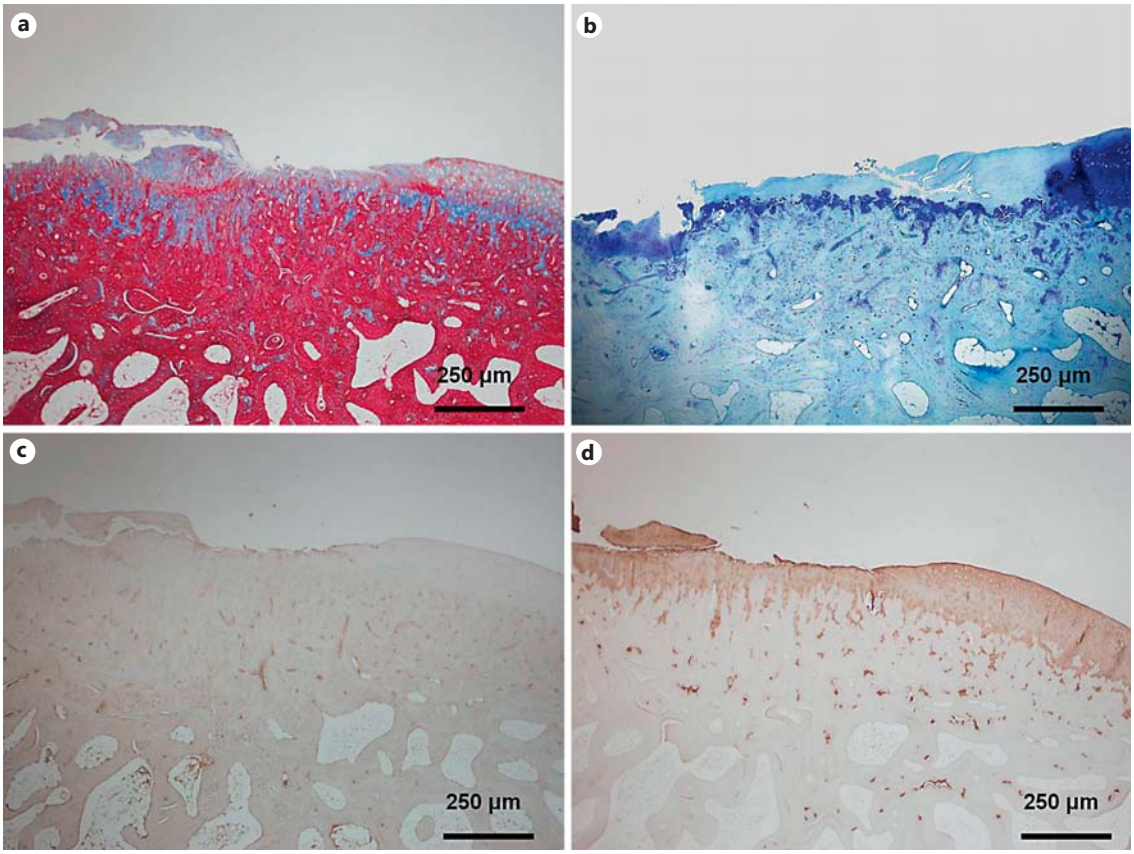

Fig. 9. Sections of regenerative cartilage in the control group. Masson's trichrome staining (**a**), toluidine blue staining (**b**), immunohistochemical staining with anti-collagen type I antibody (**c**) and immunohistochemical staining with anti-collagen type II antibody (**d**). Original magnification ×40.

Histological Observations

Histochemical Staining. In the experimental group, new cartilage was regenerated to such an extent that it was almost the same thickness as that of the adjacent articular cartilage and with a very smooth transition. The regenerative tissue showed nearly normal articular cartilage with a normal columnar pattern (fig. 8). However, in the control group where no transplantation was attempted, cartilage with a small thickness and an irregular surface was formed at the defect site (fig. 9). On toluidine blue staining, in the control group there was no staining property at the transplantation site compared with a definite color development in the adjacent normal cartilage areas. However, in the experimental group there was a profile of the color development reactions similar to that of the normal adjacent cartilage.

Immunohistochemical Staining. Immunohistochemical staining of tissue collected 12 weeks postoperatively was performed using anti-collagen types I and II antibodies. As shown in figure 8c, collagen type I was not expressed at all; however, the expression of collagen type II can be observed in figure 8d, thus indicating good articular cartilage formation. Masson's trichrome staining, toluidine blue staining, immunohistochemical staining with anti-collagen type I antibody, and immunohistochemical staining with anti-collagen type II antibody are presented in online supplementary fig. 1–4, see www.karger.com/doi/10.1159/000356488.

Discussion

There have been various surgical methods used to treat articular cartilage due to the limited regenerative potential of cartilage [Redler et al., 2012]. Among these treatments, ACI has become a standard treatment. However, it is difficult for patients to choose the optimal surgical treatment as ACI requires two surgeries for the extraction

of normal cartilage and the transplantation of cartilage following its culturing. Also, in patients in whom the articular cartilage damage is not severe and the defect area size is relatively small, ACI can be considered as possible overtreatment.

Therefore, it is necessary to review and develop the one-stage procedure. Among the previously used surgical methods, the treatments using stem cells include microfracture. As there have been many reports describing successful clinical outcomes after microfracture, new treatment modalities have been proposed [Lubowitz et al., 2007; Benthien and Behrens, 2011; Piontek et al., 2012].

Also, in cases in which a microfracture is performed, the degeneration of articular cartilage occurs over time. Accordingly, in these cases additional revision is necessary. Recent studies have shown successful experimental results with the concomitant use of a scaffold with a microfracture [Erggelet et al., 2009; Benthien and Behrens, 2011; Piontek et al., 2012].

In our experiment we performed a microfracture using a 23-guage needle which invaded the subchondral bone and which might lead to effective tissue formation with a microfracture. The transplantation of atelocollagen gel could be considered of additional use in a microfracture as it can be used as a scaffold. A scaffold might have also positively influenced cellular division, mineralization and subchondral response after microfracture.

For the procedure in which a solid-form scaffold is transplanted to the cartilage defect, the depth of the defect area should be relatively greater and the scaffold and the press fit should thus be created accordingly. In cases in which the defect areas are shallow, in order to keep the scaffold attached to the defect, a suture or membrane coverage is necessary. However, an atelocollagen and fibrin mixture do not require this process [Yamazoe et al., 2007; Choi et al., 2010; Tanaka et al., 2010]. According to the coagulation cascades, atelocollagen and fibrin mixture can be maintained at the sites where a defect is shallow. Accordingly, this mixture can easily be applied to even a posterior condyle defect to which there is no surgical access.

Collagen is the connective tissue protein which has a key role in maintaining tissue morphology, and atelocollagen is a highly purified type I collagen obtained following the treatment of pepsin from skin dermis [Magarian et al., 2012]. This atelocollagen is obtained following the removal of telopeptide which is immunologically problematic and is used for a number of purposes such as wound healing, vessel prosthesis, bone substitute and hemostatic agent [Kojima et al., 2007].

On the other hand, fibrin sealants are biological adhesives that mimic the final step of the coagulation cascade and thus help to control bone bleeding [Andrade et al., 2006]; they are also a very safe combination. Fibrin gel has recently been attracting attention as a successful substance for cartilage reconstruction. Fibrin has high biocompatibility, biodegradability, no toxicity, and has long been used as a clinical substance for bleeding control. Fibrin is also known for its successful use as an injectable carrier for generating neocartilage [Keller et al., 1985; Mawatari et al., 2006]. Fibrin can maintain the shape of an articulation for approximately 5 min after its injection, thus causing the atelocollagen to remain in the injected sites.

In our experiment, the observation period was only 12 weeks. That is because the aim of this study was to show the difference between the control group and experimental group. Twelve weeks is also enough time to compare the repaired tissues in a rabbit model [Kim et al., 2013].

In the experimental group members in whom the atelocollagen and fibrin mixture was transplanted, there was successful cartilage regeneration, and type II collagen was well expressed. The injected atelocollagen was type I collagen, but after 12 weeks of operation, the main component of regenerated tissue was type II collagen. This suggests that injected collagen gel was absorbed and bone marrow cells effectively participate in regeneration in the scaffold [Masuoka et al., 2006]. At this time, the scaffold successfully maintained the shape of the articular cartilage which was formed from the stromal cells. This phenomenon is also assumed to have a key role in making effective connectivity between cells.

The successful clinical use of this procedure can be seen during AMIC (autologous matrix-induced chondrogenesis), a procedure in which a microfracture is previously performed for damaged articular cartilage which is then covered by the collagen membrane [Peterson et al., 2002; Steinwachs et al., 2008]. However, during joint movement it is possible for a graft to detach. With the use of an atelocollagen and fibrin mixture, the defect sites were well covered, and the absence of graft detachment was confirmed though joint movement. Accordingly, in cases in which this procedure was directly applied to clinical practice, despite its application to a small lesion or in those lesions in which ACI could not be performed due to any of several reasons, and despite the presence of large-sized lesions, it is highly probable that an atelocollagen and fibrin mixture injection procedure would be a successful alternative treatment regimen for articular cartilage injuries.

Conclusion

Atelocollagen and fibrin mixture for treatment of articular cartilage defects of the knee appears to be an effective method for cartilage regeneration and also has many potential surgical advantages.

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