■ KNEE

# Single-step ultra-purified alginate gel implantation in patients with knee chondral defects

## A SINGLE-ARM CLINICAL TRIAL

**T. Onodera,**
**D. Momma,**
**M. Matsuoka,**
**E. Kondo,**
**K. Suzuki,**
**M. Inoue,**
**M. Higano,**
**N. Iwasaki**

*From Hokkaido University Hospital, Sapporo, Japan*

### Aims

**Implantation of ultra-purified alginate (UPAL) gel is safe and effective in animal osteochondral defect models. This study aimed to examine the applicability of UPAL gel implantation to acellular therapy in humans with cartilage injury.**

### Methods

**A total of 12 patients (12 knees) with symptomatic, post-traumatic, full-thickness cartilage lesions (1.0 to 4.0 cm²) were included in this study. UPAL gel was implanted into chondral defects after performing bone marrow stimulation technique, and assessed for up to three years postoperatively. The primary outcomes were the feasibility and safety of the procedure. The secondary outcomes were self-assessed clinical scores, arthroscopic scores, tissue biopsies, and MRI-based estimations.**

### Results

**No obvious adverse events related to UPAL gel implantation were observed. Self-assessed clinical scores, including pain, symptoms, activities of daily living, sports activity, and quality of life, were improved significantly at three years after surgery. Defect filling was confirmed using second-look arthroscopy at 72 weeks. Significantly improved MRI scores were observed from 12 to 144 weeks postoperatively. Histological examination of biopsy specimens obtained at 72 weeks after implantation revealed an extracellular matrix rich in glycosaminoglycan and type II collagen in the reparative tissue. Histological assessment yielded a mean overall International Cartilage Regeneration & Joint Preservation Society II score of 69.1 points (SD 10.4; 50 to 80).**

### Conclusion

**This study provides evidence supporting the safety of acellular UPAL gel implantation in facilitating cartilage repair. Despite being a single-arm study, it demonstrated the efficacy of UPAL gel implantation, suggesting it is an easy-to-use, one-step method of cartilage tissue repair circumventing the need to harvest donor cells.**

**Cite this article:** ***Bone Joint J*** **2023;105-B(8):880–887.**

## Introduction

The healing capacity of damaged cartilage is limited by the absence of nerves, vasculature, and lymphatics.[1] Bone marrow stimulation techniques (BMSTs) are the primary treatment approach for lesions < 2 cm²,[2] characterized by their technical simplicity and minimal invasiveness. Bone marrow-derived mesenchymal stem cells do not possess sufficient chondrogenic differentiation potential and are considered to result in fibrocartilage or fibrocartilaginous repair.[3] Cell-based therapies, such as autologous chondrocyte implantation (ACI), have been developed to overcome these deficits. ACI has demonstrated acceptable clinical results but with the limitation of being used primarily for defects > 4 cm².[4] In contrast, the most appropriate treatment for medium-sized (< 2 cm² to 4 cm²) lesions remains controversial. As two-step surgery with cell implantation is too invasive, less invasive techniques for medium-sized lesions are needed.

Correspondence should be sent to T. Onodera; email: tomozou@med.hokudai.ac.jp



doi:10.1302/0301-620X.105B8.BJJ-2022-1071.R2 $2.00

*Bone Joint J* 2023;105-B(8):880–887.

**Table I.** Patient inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| Traumatic cartilage injury (ICRS grade III or IV)[18] | Multiple cartilage defect |
| Osteochondral defect (Bruckl IV or V)[19] | Unrepairable ligament injury, meniscus injury |
| Aged 13 to 65 years | Remarkable subchondral bone cyst, ossification |
| Epiphyseal closure confirmed on MRI | Diagnosed with arthritis, has a history of arthritis (including osteoarthritis) |
| Isolated cartilage defect 1 to 4 $cm^2$* | Chronic ACL deficiency (> 1 year) |
| KOOS ≤ 70 points | Surgical history of cartilage defect |
| | Walking impairment due to disease |
| | Pregnant, lactating, or possibly pregnant |
| | Serious cardiac, hepatic, renal, haematological, or endocrine complications |

*The calculation is defined as: long radius × short radius × 3.14 ($cm^2$).
ACL, anterior cruciate ligament; ICRS, International Cartilage Regeneration and Joint Preservation Society; KOOS, Knee injury and Osteoarthritis Outcome Score.

**Table II.** Baseline characteristics of patients.

| Characteristic | Total |
|---|---|
| Patients, n | 12 |
| Mean age, yrs (range) | 33.1 (16 to 48) |
| **Sex, n** | |
| Male | 8 |
| Female | 4 |
| Mean BMI, $kg/m^2$ (SD; range) | 24.47 (2.98; 20.5 to 29.4) |
| **Pathogenesis, n** | |
| Cartilage injury | 10 |
| Osteochondral defect | 2 |
| **Mean defect size by location, cm2 (SD; range)** | |
| MFC* | 1.50 (0.42; 1.1 to 2.5) |
| LFC† | 1.65 (0.78: 1.1 to 2.2) |
| Femoral trochlea‡ | 2.50 (0.14; 1.0 to 2.6) |
| Patella§ | 2.00 (1.27; 1.2 to 2.9) |
| Mean dotal defect size, $cm^2$ (SD; range) | 1.81 (0.70; 1.0 to 2.9) |
| Mean size after debridement, $cm^2$ (SD; range) | 1.83 (0.83; 1.0 to 3.8) |
| **Stage, n** | |
| ICRS grade III | 8 |
| ICRS grade IV | 2 |
| Bruckl stage V | 2 |
| **Additional treatment, n** | |
| ACL reconstruction | 2 |
| High tibial osteotomy | 1 |
| Meniscus repair | 2 |

*n = 5.
†n = 2.
‡n = 3.
§n = 2.
ACL, anterior cruciate ligament; ICRS, International Cartilage Regeneration and Joint Preservation Society; LFC, lateral femoral condyle; MFC, medial femoral condyle; SD, standard deviation.

Several matrix-induced chondrogenic techniques have recently been developed and have demonstrated acceptable clinical results for medium-sized lesions.[5-8] These methods are potential strategies for treating medium-sized lesions via a single-step procedure. Alginate gels are biocompatible materials used to replace chondral defects and have demonstrated biochemical characteristics similar to native hyaline cartilage.[9-11] The advantage of alginate gel is that it can be stabilized in situ without additional fixation material, such as biodegradable pins, resorbable sutures, and glue,[12-14] thus making autologous matrix-induced chondrogenesis a less invasive technique. We developed an injectable ultra-purified alginate (UPAL) gel for use as a bioactive scaffold for cartilage tissue regeneration, and demonstrated that BMSTs augmented with UPAL gel enhance cartilage repair in rabbit and canine models.[15-17] We hypothesized that BMSTs augmented with UPAL gel could also induce hyaline-like cartilage repair in humans. This study aimed to assess whether acellular UPAL gel implantation is a safe technique to augment articular cartilage repair.

## Methods

**Study design and patients.** Between January 2016 and April 2017, 12 patients (eight male and four female; age range 16 to 45 years) with isolated full-thickness cartilage defects of the knee (1 $cm^2$ to 4 $cm^2$; International Cartilage Regeneration & Joint Preservation Society (ICRS)[18] grade III or IV) were enrolled. Table I lists the inclusion and exclusion criteria for patient selection and Table II shows the patient characteristics. Clinical outcome and imaging data were collected. Images were evaluated by a radiologist (KS) with > ten years' experience in joint MRI interpretation who was blinded to the patient data, intraoperative findings, and postoperative periods. Each image was evaluated twice to minimize variability.

**Preparation of alginate gel.** An in-situ forming material based on UPAL gel (Mochida Pharmaceutical, Japan) with a molecular weight of 1,700 kDa was used in this study. The material was filter-sterilized (pore size 0.22 mm) and subsequently freeze-dried for packaging in a sterile vial. The purified alginate had a considerably low endotoxin level (5.76 EU/g vs 75,950 EU/g used for commercially available grade alginate (sodium alginate 500, 199-09961; Wako, Japan)). In this study, 2% w/v sodium alginate solution dissolved in normal saline was used.

**Surgical procedure.** Arthroscopic evaluation and UPAL gel implantation surgery through a small arthrotomy were performed (Supplementary Figure a) in a single procedure. First, the cartilage defect was visualized and debrided down to the subchondral bone and healthy surrounding cartilage using a sharp blade. For the bone marrow stimulation technique, several holes (diameter 1.0 mm) were drilled into the defect. After debridement and bone marrow stimulation of the cartilage lesion, the defects were filled with a 2% sodium alginate solution (Supplementary Figure aa). The alginate surface layer was subsequently gelated with $CaCl_2$ five minutes after injection to avoid overexposure to cytotoxic $CaCl_2$ (Supplementary Figure ab). Next, the knee joint

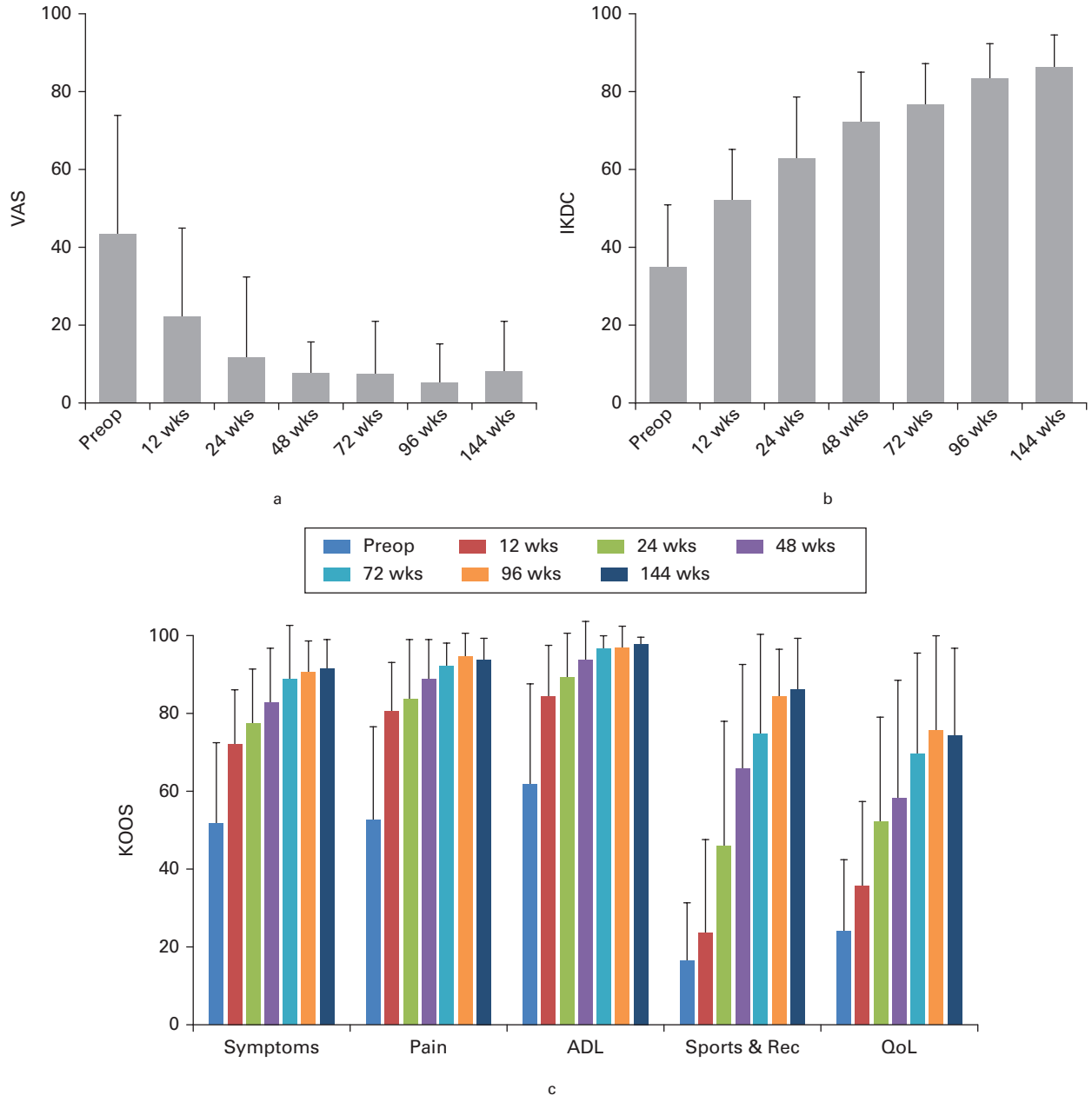



Fig. 1

Patient clinical scores. a) Visual analog scale; b) International Knee Documentation Committee (IKDC); and c) Knee injury and Osteoarthritis Outcome Score (KOOS) subscores. The Wilcoxon signed-rank test was used for data collected at different follow-up timepoints. All $p < 0.05$ vs preoperative scores (Wilcoxon signed-rank test). ADL, activities of daily living; QoL, quality of life; VAS, visual analogue scale.

was irrigated with normal saline. To confirm the stability of the implanted UPAL gel, the affected knee was flexed and extended intraoperatively to return the patella to its original position. Postoperative rehabilitation consisted of non-weightbearing exercise in which extension fixation was applied for the first two weeks. Partial weightbearing and range of motion exercises with a continuous passive motion were initiated at two weeks postoperatively. Finally, full weightbearing exercise was allowed at four weeks postoperatively.

**Outcome measures.** All adverse events, local and systemic reactions, and lab results were recorded. All patients were assessed on the day of the operation and UPAL gel implantation as well as at two, four, 12, 24, 48, 72, 96, and 144 weeks postoperatively, and their general and knee conditions were examined. General laboratory findings, including the leucocyte counts and serum CRP levels, visual analogue scale (VAS), and Knee injury and Osteoarthritis Outcome Scores (KOOS),[20] were assessed at the same timepoints. MRI was performed using a 3.0

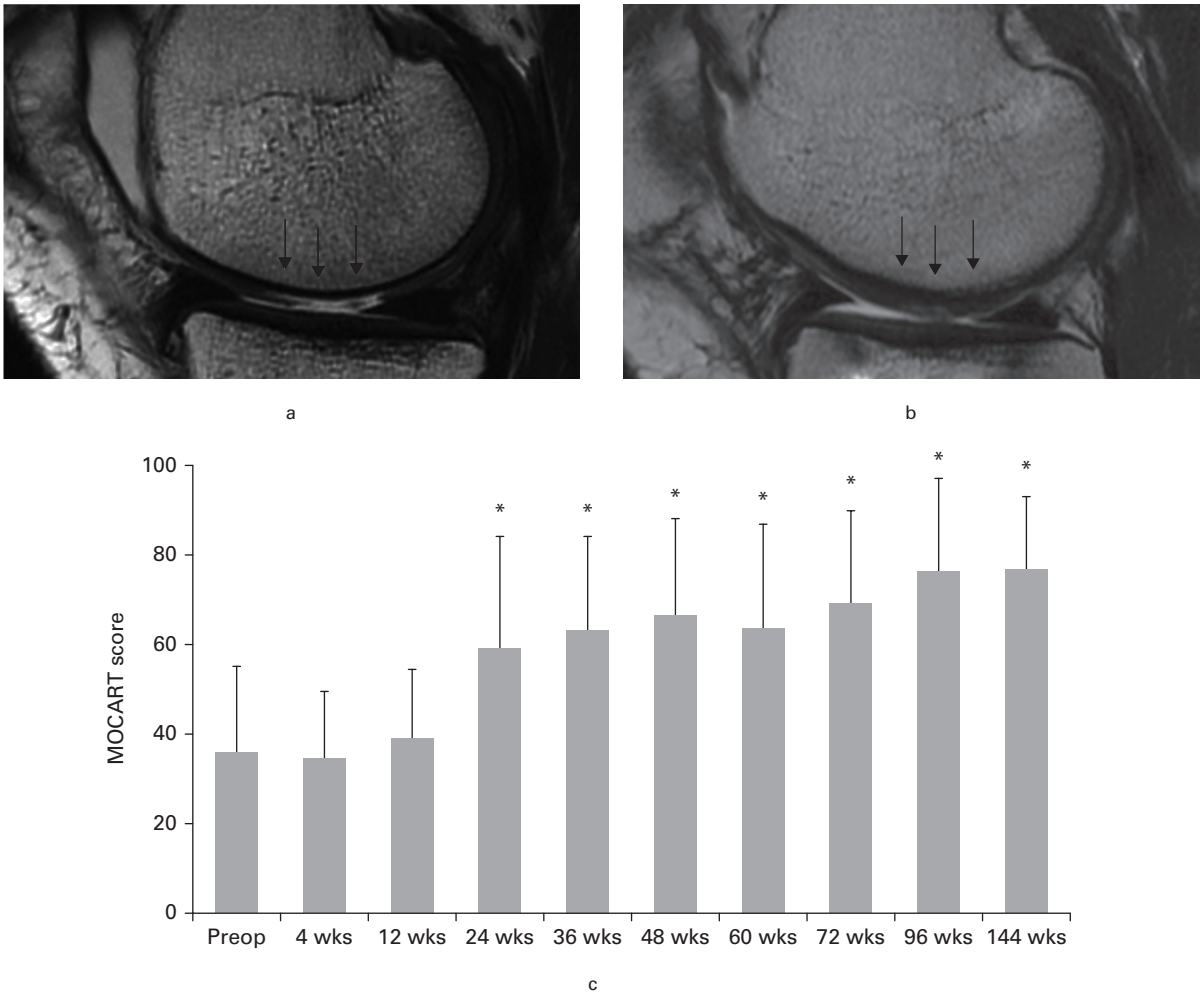



Fig. 2

Representative MRI findings a) preoperatively and b) 72 weeks postoperatively. The black arrows indicate a) cartilage defect and b) the repair site following ultra-purified alginate gel + implantation. c) The Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score indicates that the cartilage defects treated with ultra-purified alginate gel were gradually filled with newly generated tissue that matured over time. *p < 0.05 versus four weeks (Wilcoxon signed-rank test).

T MRI machine (Ingenia Elition 3.0 T; Philips Healthcare, the Netherlands) preoperatively and at four, 12, 24, 36, 48, 60, 72, 96, and 144 weeks postoperatively. MRI evaluation focused on evidence of graft failure, such as migration or displacement, and the presence of fluid surrounding the graft on T2-weighted images. Cartilage surface characteristics highlighted fluid in the joint, and oedema in the subchondral bone were evaluated using fat saturation and proton density-weighted imaging. The quality of repair of the joint surface was evaluated using the magnetic resonance observation of cartilage repair tissue (MOCART)[21] scoring system preoperatively and at four, 12, 24, 36, 48, 60, 72, 96, and 144 weeks postoperatively.

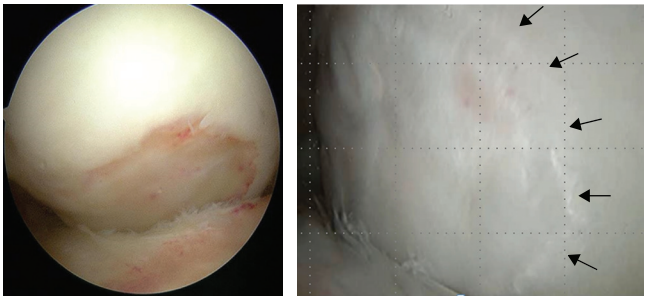
At 72 weeks, second-look arthroscopy was performed on 11 patients, and a needle biopsy sample was obtained from the centre of the reparative tissue after obtaining patient consent. This procedure was performed after an MRI examination at 72 weeks to avoid influencing the MRI assessment. One patient persistently refused a biopsy.

Sections of biopsy specimens were stained with Safranin O and haematoxylin and eosin to assess histological features, based on the ICRS II scoring system.[18] All data analyses were performed in a blinded manner by independent researchers (TO and MM for histological analyses and KS for MRI analyses).

**Statistical analysis.** After determining the normality of the data distributions, the Wilcoxon signed-rank test was used for data collected at different follow-up timepoints. To investigate changes in clinical and MRI-based outcomes over time, one-way repeated-measures analysis of variance was applied. Data were analyzed using JMP software v. 14 (SAS Institute, USA), and statistical significance was set at $p < 0.05$.

## Results

No material-derived or severe adverse events occurred during the follow-up period. All patients were followed up for > three years postoperatively, and no additional treatment was required during this period. The mean size of the osteochondral defect



a b

Fig. 3

Representative arthroscopic findings. Arthroscopic image a) before ultra-purified alginate implantation and b) during second-look arthroscopy 72 weeks postoperatively. The defects were covered with regenerative cartilage-like tissue at 72 weeks. Arrows indicate the border between regenerating and healthy cartilage.

was 1.83 $cm^2$ (1.0 to 3.8). The site of the osteochondral defect was the medial femoral condyle, lateral femoral condyle, femoral trochlea, and patella in five, two, three, and two cases, respectively.

**Clinical outcomes and MRI findings.** The mean VAS, International Knee Documentation Committee subjective form,[22] and five KOOS subscores all improved significantly from preoperative to three years postoperatively (Figure 1). Based on MRI assessments, cartilage defects filled with new tissue over time (Figures 2a and 2b), and defects were confirmed to have been repaired in all patients without detectable hypertrophy within 72 weeks. In the analysis of the MOCART score, integration into the border zone, filling with repair tissue, surface, and signal intensity all improved over time (Figure 2c). The MOCART score showed significant improvement between 24 weeks and three years after UPAL gel implantation compared to that obtained preoperatively, with repair tissue gradually maturing (preoperative 34.6 (standard deviation (SD) 15.1) vs postoperative three years 76.8 (SD 16.2); $p = 0.001$, Wilcoxon signed-rank test).

**Arthroscopic findings.** In total, 11 of the 12 patients underwent second-look surgery at 72 weeks postoperatively (ranging between 71 and 81; Figure 3). Arthroscopic examinations consistently demonstrated improvements in the lesion ICRS grade from III or IV to I or II, except in one case classified as grade III on both observations. In addition, no hypertrophy was observed in the regenerative cartilage of 11 patients. No synovitis or inflammation was observed during second-look surgery in any of the 11 patients.

**Histological findings.** The reparative tissues exhibited a smooth surface characterized by sufficient chondrocytes with cartilage lacunae, except for the superficial layer of the defect (Figure 4). The collagen orientation identified vertically oriented deep zones in the sections of the reparative tissue. Vertically oriented collagen fibres in the deep zone exhibited favourable integration with the underlying subchondral bone (Supplementary Figure b). Based on these findings, the ICRS II subscores, such as tissue morphology, basal integration, and mid/deep zone assessment, were high (Figure 5).

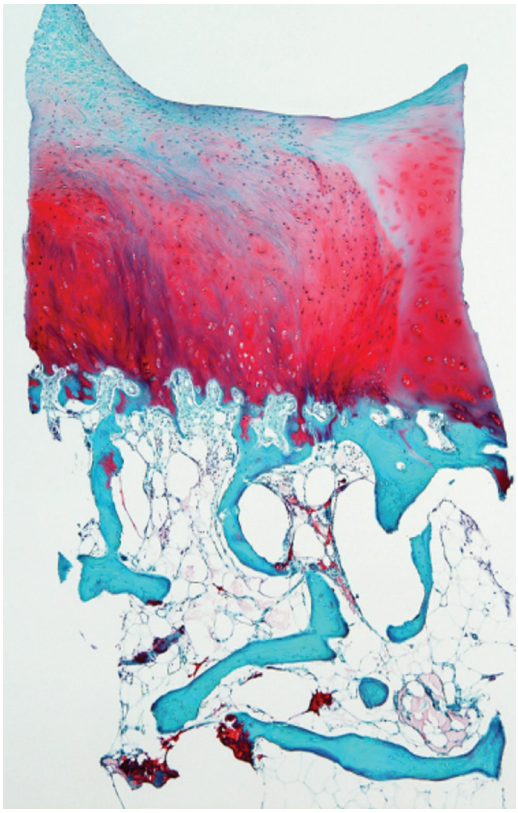


Fig. 4

Histological analysis of the representative biopsy specimens obtained at 72 weeks postoperatively. The sections were stained with Safranin O and observed at ×4 magnification.

## Discussion

This study with a three-year follow-up period found the use of UPAL gel for the clinical repair of articular cartilage defects in knee joints is safe and feasible. UPAL gel potentially improves cell proliferation and cartilage differentiation for BMSTs, resulting in significantly improved defect filling and self-assessment scores. Histological and MRI analyses established satisfactory clinical outcomes and a gradual improvement in the quality of repair tissue over time.

UPAL gel exhibits substantial biocompatibility by reducing the cytotoxicity of alginic acid, and is achieved by a simple, rapid, cell-free procedure. Alginic acid displays a high affinity for $Ca^{2+}$ ions, which are divalent cations, and gelation occurs immediately when ionized alginic acid comes into contact with Ca. Impurities in conventional alginate have limited clinical applications, however, UPAL gel, a highly purified, biocompatible alginate material, exhibits high cartilage regeneration by dramatically reducing the endotoxin levels in animal models.[9,23] The current clinical, radiological, and arthroscopic findings were not associated with any obvious adverse effects in any of the 12 patients. This suggests that a safety profile similar

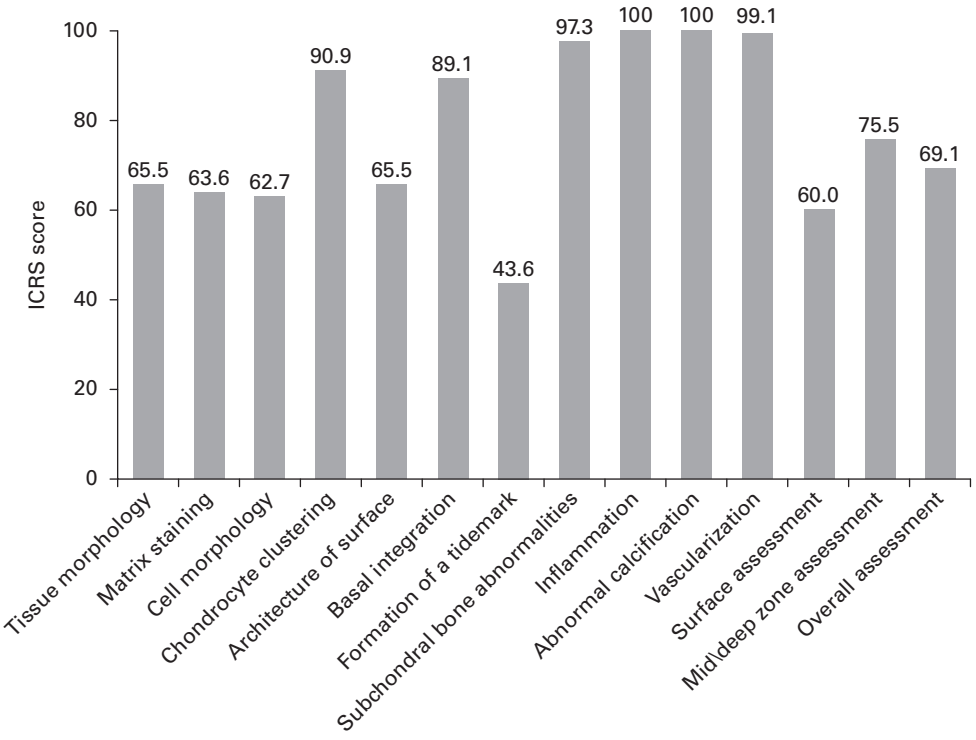



Fig. 5

Histological assessment of the biopsy specimens obtained at 72 weeks post implantation using the International Cartilage Repair Society (ICRS) II scoring system.

to that of previous animal experimental data was validated in this trial.[16]

The histological results from this study showed the overall quality of the repaired tissue was similar to that obtained in our preclinical canine studies.[15,17] This study used a single-arm study design, meaning no comparisons of histological evaluation were made. Comparison with a previous study found scores were higher in the deep layers of cartilage tissue for factors, such as cell morphology, mid-/deep-zone assessment, subchondral bone, and tidemarks.[24] Studies have reported the presence of a hyaline-like cartilage matrix in the middle-to-deep layers of cellular cartilage regeneration but no reports have described cartilage regeneration in the absence of cell implantation.[25,26] Consistently high occupancy by hyaline-like cartilage substrates has not been demonstrated in previously reported clinical studies on cell-based cartilage repair.[26–29] The current histological scores for UPAL gel implantation were comparable or superior to those of previous high-quality human trials.[30–32] The close connection between bone marrow fluid and alginate in the zol-state might have promoted regeneration in the middle/deep zone and integration between the reparative tissue and subchondral bone. Since only the surface in contact with $CaCl_2$ forms a gel, while the inside remains in a solvent state, cells and growth factors could theoretically interact, leading to the healing of hyaline-like cartilage. Observation of UPAL during surgery showed internal bleeding confirmed by encapsulation in the cartilage defect. Based on these reasons, the reparative tissue was considered to show favourable histological characteristics.

We have previously reported that UPAL gel implantation resulted in regenerated cartilage tissues with favourable collagen orientation.[15] The collagen orientation is important for cartilage load-bearing capacity.[33,34] Although the chondrocytes used for ACI are cultured ex vivo and are available in large quantities, the mechanical loading required for collagen fibre orientation may be deficient in ex vivo cultures. Our results suggest that physiological loading conditions in vivo enhanced the chondrogenic potential of undifferentiated bone marrow cells in situ, leading to organized reparative tissues and in turn collagen fibre orientation.

From previous results, one concern is that insufficient repair of the subchondral bone potentially results in deficient mechanical properties compared with normal cartilage.[15,17] Reports have described cell-based treatments for enhancing subchondral bone repair.[35,36] In contrast, UPAL gel implantation can promote endogenous cartilage repair and inhibit hypertrophic changes in recruited cells detrimental to osteogenesis, whereas subchondral bone repair may be inadequate.[9] When severe subchondral bone loss is diagnosed preoperatively, adjuvant therapies, such as bone grafting or cell implantation, should be considered.

The main drawback of ACI is the requirement for two-stage invasive surgery.[4,37] Favourable clinical results from one-stage treatment with bone marrow aspirate concentrate (BMAC) have recently been reported, citing it as a cell therapy that overcomes

these drawbacks.[11] BMAC requires in vitro concentration treatment and is expensive. The current strategy, as a one-step approach, has the potential to overcome these limitations. In addition, UPAL gel can be stabilized in situ if the cartilage defect can be maintained horizontally for a brief period during surgery. Determining the appropriate surgical indication for this technique and establishing an arthroscopic method based on UPAL gel will lead to BMSTs emerging as valuable and cost-effective clinical techniques.

This study has several limitations. The first limitation concerns sample heterogeneity, the relatively small number of enrolled patients, and lack of a control group. In this study, there were cases in which high tibial osteotomy and anterior cruciate ligament reconstruction were performed in combination. These surgeries may affect postoperative outcomes however, they often involve UPAL gel implantation and were performed safely as a pilot procedure. Second, our outcomes were investigated for only three years. Longer follow-up periods are needed to further support the proposed treatment. Third, arthrotomy was performed as a treatment in this series. A recent report found that the mid-term outcomes of arthroscopic treatment were equivalent to those of mini-open procedures,[38] meaning less invasive arthroscopic techniques should be developed in the future. Further comparative and long-term studies are required to test its possible superiority over conventional treatments.

In conclusion, in this human clinical trial, BMSCs augmented with UPAL gel induced hyaline-like cartilage repair. This method is a one-step, minimally invasive cartilage regenerative treatment that avoids harvesting donor cells and expands the surgical indications for BMSTs without loss of technical simplicity or cost-effectiveness.

### Take home message

- This study demonstrates that acellular ultra-purified alginate gel implantation is a safe means of augmenting articular cartilage repair.

## Twitter

Follow the authors @hokudai_seikei

## Supplementary material

Figures showing schematic representation of ultra-purified alginate gel implantation, representative surgical photos, and histology of the representative biopsy specimens.

## References


1. **Sophia Fox AJ**, **Bedi A**, **Rodeo SA**. The basic science of articular cartilage: structure, composition, and function. *Sports Health*. 2009;1(6):461–468.
2. **Mandelbaum BR**, **Browne JE**, **Fu F**, **et al**. Articular cartilage lesions of the knee. *Am J Sports Med*. 1998;26(6):853–861.
3. **Mithoefer K**, **Williams RJ**, **Warren RF**, **et al**. The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. *J Bone Joint Surg Am*. 2005;87-A(9):1911–1920.
4. **Bartlett W**, **Skinner JA**, **Gooding CR**, **et al**. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. *J Bone Joint Surg Br*. 2005;87-B(5):640–645.
5. **Benthien JP**, **Behrens P**. Autologous matrix-induced chondrogenesis (AMIC). A one-step procedure for retropatellar articular resurfacing. *Acta Orthop Belg*. 2010;76(2):260–263.
6. **Gille J**, **Reiss E**, **Freitag M**, **et al**. Autologous matrix-induced chondrogenesis for treatment of focal cartilage defects in the knee: a follow-up study. *Orthop J Sports Med*. 2021;9(2):2325967120981872.
7. **Schiavone Panni A**, **Del Regno C**, **Mazzitelli G**, **D'Apolito R**, **Corona K**, **Vasso M**. Good clinical results with autologous matrix-induced chondrogenesis (AMIC) technique in large knee chondral defects. *Knee Surg Sports Traumatol Arthrosc*. 2018;26(4):1130–1136.
8. **Jun Z**, **Yuping W**, **Yanran H**, **et al**. Human acellular amniotic membrane scaffolds encapsulating juvenile cartilage fragments accelerate the repair of rabbit osteochondral defects. *Bone Joint Res*. 2022;11(6):349–361.
9. **Igarashi T**, **Iwasaki N**, **Kasahara Y**, **Minami A**. A cellular implantation system using an injectable ultra-purified alginate gel for repair of osteochondral defects in a rabbit model. *J Biomed Mater Res A*. 2010;94(3):844–855.
10. **Igarashi T**, **Iwasaki N**, **Kawamura D**, **et al**. Repair of articular cartilage defects with a novel injectable in situ forming material in a canine model. *J Biomed Mater Res A*. 2012;100(1):180–187.
11. **Xu L**, **Urita A**, **Onodera T**, **et al**. Ultrapurified alginate gel containing bone marrow aspirate concentrate enhances cartilage and bone regeneration on osteochondral defects in a rabbit model. *Am J Sports Med*. 2021;49(8):2199–2210.
12. **Fossum V**, **Hansen AK**, **Wilsgaard T**, **Knutsen G**. Collagen-covered autologous chondrocyte implantation versus autologous matrix-induced chondrogenesis: a randomized trial comparing 2 methods for repair of cartilage defects of the knee. *Orthop J Sports Med*. 2019;7(9):2325967119868212.
13. **Gille J**, **Meisner U**, **Ehlers EM**, **Müller A**, **Russlies M**, **Behrens P**. Migration pattern, morphology and viability of cells suspended in or sealed with fibrin glue: a histomorphologic study. *Tissue Cell*. 2005;37(5):339–348.
14. **Petersen W**, **Zelle S**, **Zantop T**. Arthroscopic implantation of a three dimensional scaffold for autologous chondrocyte transplantation. *Arch Orthop Trauma Surg*. 2008;128(5):505–508.
15. **Baba R**, **Onodera T**, **Matsuoka M**, **et al**. Bone marrow stimulation technique augmented by an ultrapurified alginate gel enhances cartilage repair in a canine model. *Am J Sports Med*. 2018;46(8):1970–1979.
16. **Baba R**, **Onodera T**, **Momma D**, **et al**. A novel bone marrow stimulation technique augmented by administration of ultrapurified alginate gel enhances osteochondral repair in a rabbit model. *Tissue Eng Part C Methods*. 2015;21(12):1263–1273.
17. **Onodera T**, **Baba R**, **Kasahara Y**, **Tsuda T**, **Iwasaki N**. Therapeutic effects and adaptive limits of an acellular technique by ultrapurified alginate (UPAL) gel implantation in canine osteochondral defect models. *Regen Ther*. 2020;14:154–159.
18. **Mainil-Varlet P**, **Van Damme B**, **Nesic D**, **Knutsen G**, **Kandel R**, **Roberts S**. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. *Am J Sports Med*. 2010;38(5):880–890.
19. **Brückl R**, **Rosemeyer B**, **Thiermann G**. Osteochondrosis dissecans of the knee. Results of operative treatment in juveniles. *Arch Orthop Trauma Surg (1978)*. 1984;102(4):221–224.
20. **Roos EM**, **Lohmander LS**. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. *Health Qual Life Outcomes*. 2003;1:64.
21. **Welsch GH**, **Zak L**, **Mamisch TC**, **Resinger C**, **Marlovits S**, **Trattnig S**. Three-dimensional magnetic resonance observation of cartilage repair tissue (MOCART) score assessed with an isotropic three-dimensional true fast imaging with steady-state precession sequence at 3.0 Tesla. *Invest Radiol*. 2009;44(9):603–612.
22. **Irrgang JJ**, **Anderson AF**, **Boland AL**, **et al**. Development and validation of the international knee documentation committee subjective knee form. *Am J Sports Med*. 2001;29(5):600–613.
23. **Sukegawa A**, **Iwasaki N**, **Kasahara Y**, **Onodera T**, **Igarashi T**, **Minami A**. Repair of rabbit osteochondral defects by an acellular technique with an ultrapurified alginate gel containing stromal cell-derived factor-1. *Tissue Eng Part A*. 2012;18(9–10):934–945.
24. **Méthot S**, **Changoor A**, **Tran-Khanh N**, **et al**. Osteochondral biopsy analysis demonstrates that BST-CarGel treatment improves structural and cellular characteristics of cartilage repair tissue compared with microfracture. *Cartilage*. 2016;7(1):16–28.
25. **Shimomura K**, **Hamada H**, **Hart DA**, **et al**. Histological analysis of cartilage defects repaired with an autologous human stem cell construct 48 weeks postimplantation reveals structural details not detected by T2-mapping MRI. *Cartilage*. 2021;13(1_suppl):694S–706S.

26. **Mumme M**, **Barbero A**, **Miot S**, **et al**. Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. *Lancet*. 2016;388(10055):1985–1994.
27. **Gobbi A**, **Karnatzikos G**, **Scotti C**, **Mahajan V**, **Mazzucco L**, **Grigolo B**. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: results at 2-year follow-up. *Cartilage*. 2011;2(3):286–299.
28. **Sekiya I**, **Muneta T**, **Horie M**, **Koga H**. Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects. *Clin Orthop Relat Res*. 2015;473(7):2316–2326.
29. **de Windt TS**, **Vonk LA**, **Slaper-Cortenbach ICM**, **et al**. Allogeneic mesenchymal stem cells stimulate cartilage regeneration and are safe for single-stage cartilage repair in humans upon mixture with recycled autologous chondrons. *Stem Cells*. 2017;35(1):256–264.
30. **Hoemann CD**, **Tran-Khanh N**, **Chevrier A**, **et al**. Chondroinduction is the main cartilage repair response to microfracture and microfracture with BST-CarGel: results as shown by ICRS-II histological scoring and a novel zonal collagen type scoring method of human clinical biopsy specimens. *Am J Sports Med*. 2015;43(10):2469–2480.
31. **Jo CH**, **Lee YG**, **Shin WH**, **et al**. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells*. 2014;32(5):1254–1266.
32. **de Windt TS**, **Vonk LA**, **Slaper-Cortenbach ICM**, **Nizak R**, **van Rijen MHP**, **Saris DBF**. Allogeneic MSCs and recycled autologous chondrons mixed in a one-stage cartilage cell transplantion: a first-in-man trial in 35 patients. *Stem Cells*. 2017;35(8):1984–1993.
33. **Jeffery AK**, **Blunn GW**, **Archer CW**, **Bentley G**. Three-dimensional collagen architecture in bovine articular cartilage. *J Bone Joint Surg Br*. 1991;73-B(5):795–801.
34. **Buckley MR**, **Gleghorn JP**, **Bonassar LJ**, **Cohen I**. Mapping the depth dependence of shear properties in articular cartilage. *J Biomech*. 2008;41(11):2430–2437.
35. **Veronesi F**, **Cadossi M**, **Giavaresi G**, **et al**. Pulsed electromagnetic fields combined with a collagenous scaffold and bone marrow concentrate enhance osteochondral regeneration: an in vivo study. *BMC Musculoskelet Disord*. 2015;16:233.
36. **Caminal M**, **Peris D**, **Fonseca C**, **et al**. Cartilage resurfacing potential of PLGA scaffolds loaded with autologous cells from cartilage, fat, and bone marrow in an ovine model of osteochondral focal defect. *Cytotechnology*. 2016;68(4):907–919.
37. **Teo AQA**, **Wong KL**, **Shen L**, **et al**. Equivalent 10-year outcomes after implantation of autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation for chondral defects of the knee. *Am J Sports Med*. 2019;47(12):2881–2887.
38. **Schagemann J**, **Behrens P**, **Paech A**, **et al**. Mid-term outcome of arthroscopic AMIC for the treatment of articular cartilage defects in the knee joint is equivalent to mini-open procedures. *Arch Orthop Trauma Surg*. 2018;138(6):819–825.



**Author information:**
T. Onodera, MD, PhD, Orthopaedic Surgeon, Lecturer
N. Iwasaki, MD, PhD, Professor, Orthopaedic Surgeon
Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan; Global Station of Soft Matter, Global Institution for Collaborative Research and Education, Sapporo, Japan.

D. Momma, MD, PhD, Assistant Professor, Orthopaedic Surgeon, Center for Sports Medicine, Hokkaido University Hospital, Sapporo, Japan.

M. Matsuoka, MD, PhD, Assistant Professor, Orthopaedic Surgeon, Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan.

E. Kondo, MD, PhD, Professor, Orthopaedic Surgeon, Center for Sports Medicine, Hokkaido University Hospital, Sapporo, Japan; Hokkaido Orthopaedic Memorial Hospital, Sapporo, Japan.

K. Suzuki, MD, PhD, Orthopaedic Surgeon, Hokkaido Orthopaedic Memorial Hospital, Sapporo, Japan.

M. Inoue, MD, PhD, Orthopaedic Surgeon, NTT-East Sapporo Hospital, Sapporo, Japan.

M. Higano, BS, Development Manager, Mochida Pharmaceutical Co. Ltd, Tokyo, Japan.



**Author contributions:**
T. Onodera: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing.
M. Daisuke: Data curation, Writing – review & editing.
M. Matsuoka: Data curation, Writing – review & editing.
E. Kondo: Data curation, Writing – review & editing.
K. Suzuki: Data curation, Writing – review & editing.
M. Inoue: Data curation, Writing – review & editing.
M. Higano: Conceptualization, Methodology, Formal analysis, Data curation.
N. Iwasaki: Conceptualization, Supervision.

**Funding statement:**
The authors received no financial for the research, authorship, and/or publication of this article.

**Data sharing:**
The data that support the findings for this study are available to other researchers from the corresponding author upon reasonable request.

**Acknowledgements:**
The authors are especially thankful to Dr Keita Sakamoto, Nozomu Watanabe, Toshiya Endo, and Mochida Pharmaceutical Co Ltd, a participating company in NexTEP, for material preparations.

**Ethical review statement:**
This study was conducted in accordance with the principles of the Declaration of Helsinki, the International Committee on Harmonization Good Clinical Practice Guidelines, and applicable local laws and regulations. This observational human study, limited to 12 cases, was approved by the Hokkaido University Institutional Review Board (No.15056). Informed consent was obtained from all patients prior to their enrolment in the study.

**Open access funding:**
The authors confirm that the open access fee was self-funded.




This article was primary edited by P. Walmsley.