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Heparin-Conjugated Fibrin Hydrogel with Chondroinductive Growth Factors and Human Synovium-Derived Mesenchymal Stem Cells for the Treatment of Articular Cartilage Defects: Evaluation of Clinical Safety

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Abstract

The purpose of this study was to evaluate the safety of an injectable heparin-conjugated fibrin (HCF) hydrogel containing human synovium-derived mesenchymal stem cells (SDMSCs), TGF-β1, and BMP-4 after implantation into articular cartilage defect in patients with osteoarthritis (OA). The study included 15 OA patients with a mean age of 44.2±18.0 years. The median articular cartilage defect size was 4.9±2.0 cm. HCF hydrogel, containing SDMSCs and growth factors (TGF-β1 and BMP-4), was implanted into the cartilage defect using DUPLOJECT two-syringe device connected with the DUPLOTIP dual lumen cannula. Clinical and radiological outcomes were evaluated with VAS, WOMAC, KOOS, and MOCART. The clinical study results showed that implantation of HCF hydrogel with autologous SDMSCs, TGF-β1, and BMP-4 appeared to be safe and did not show severe adverse events in OA patients. The assessment of clinical outcomes after 6 months showed improvement in VAS, WOMAC, and KOOS scores in all patients. The MOCART evaluation demonstrated an enhancement of cartilage tissue repair in 73.3% of OA patients at 6 months after surgery. Thus, implantation of HCF hydrogel with SDMSCs, TGF-β1, and BMP-4 was safe and demonstrated signs of improvement in articular cartilage repair. The evaluation of the long-term safety and therapeutic efficacy of HCF hydrogel is required in a further clinical study using a larger number of OA patients. (**International Journal of Biomedicine. 2022;12(4):539-547.**).

Keywords: mesenchymal stem cells • fibrin hydrogel • growth factors • cartilage defect • clinical safety

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Abbreviations

ADL, activities of daily living; **AEs**, adverse events; **BMP**, bone morphogenetic proteins; **ECM**, extracellular matrix; **HCF**, heparin-conjugated fibrin; **ICRS**, International Cartilage Repair Society; **KOOS**, Knee Injury and Osteoarthritis Outcome Score; **LMWH**, low-molecular-weight heparin; **MOCART**, Magnetic Resonance Observation of Cartilage Repair Tissue; **MSCs**, mesenchymal stem cells; **MRI**, magnetic resonance imaging; **OA**, osteoarthritis; **QoL,** Quality of Life; **SDMSCs**, synovium-derived mesenchymal stem cells; **SAEs**, severe adverse events; **TGF-β**, transforming growth factor-beta; **VAS**, Visual Analogue Scale; **WOMAC**, Western Ontario and McMaster Universities Osteoarthritis Index.

Introduction

Osteoarthritis (OA) is the most common chronic progressive joint disease, which is a serious general medical and social problem, resulting in substantial economic costs due to the high prevalence and severity of damage to the musculoskeletal system.[1,2] According to WHO, more than 300 million people in 195 countries suffer from this disease, while there is a constant increase in one of the indicators of OA – "the number of years lived by the population in a state of disability."[3] Current methods of managing articular cartilage defects include multiple microfractures, osteochondral autograft transfer, osteochondral allograft transplantation, autologous chondrocyte implantation, and matrix-assisted autologous chondrocyte implantation.[4] However, these clinical repair methods mainly lead to short-term functional regeneration with the formation of fibrocartilage and cannot provide sustainable restoration of functional hyaline cartilage.[5,6] Currently, tissue engineering technology using various hydrogel scaffolds, mesenchymal stem cells (MSCs), and growth factors is considered as the most promising therapeutic strategy for the regeneration of cartilage and osteochondral defects.[7,8] MSCs are multipotent stem cells that possess self-renewal capacity and can differentiate into various specialized cell types such as adipocytes, chondrocytes, and osteoblasts.[9,10] MSCs present in many tissues of the human body. Recent studies have revealed that MSCs can be isolated from the synovial membrane.[11,12] One of the advantages of synovium-derived MSCs (SDMSCs) is that these cells are tissue-resident stem cells, which actively participate in maintaining joint homeostasis and cartilage repair.[13,14] Moreover, it has been demonstrated that compared to MSCs isolated from other tissue sources, SDMSCs have greater proliferation activity and chondrogenic potential in vitro, rendering SDMSCs as an appropriate source for cartilage regeneration.[15,16]

It was reported that the implantation of MSCs alone often leads to the formation of fibrocartilage, indicating that the in vivo environment is not sufficient to induce chondrogenesis in the cartilage defect.[17] To induce chondrogenesis in MSCs and hyaline cartilage formation, specific growth factors such as TGF-β1, BMP-2, BMP-4, and IGF-1 are required.[6,18] Recently, several studies demonstrated that MSCs encapsulated in hydrogel scaffolds with chodroinductive growth factors significantly repaired cartilage defects in contrast to individual applications of MSCs or growth factors.[19] For example, Gugjoo et al.[20] showed that implantation of laminin gel scaffold containing MSCs with IGF-1 and TGF-β1 significantly promoted the regeneration of damaged hyaline cartilage and subchondral bone tissue in rabbit osteochondral model. Implantation of the hybrid scaffold composed of chitosan hydrogel and a decellularized bone matrix for co-delivery of rabbit SDMSCs and TGF-β1 promoted chondrogenic differentiation of loaded SDMSCs and significantly enhanced cartilage regeneration.[21] Rabbit adipose-derived MSCs encapsulated in injectable PLGA hydrogels with BMP-2 significantly improved cartilage repair in chondral defects except in untreated controls and microfracture treatment alone.[22]

Recently, we have developed an injectable heparin-conjugated fibrin (HCF) hydrogel containing SDMSCs and two chondroinductive factors (TGF-β1 and BMP-4) for the regeneration of osteochondral articular defect.[23] Our in vitro study showed that HCF hydrogel has good biocompatibility with encapsulated SDMSCs and the ability to control the release of TGF-β1 and BMP-4 for 4 weeks. A preclinical study revealed that implantation of SDMSCs in HCF hydrogel in combination with TGF-β1 and BMP-4 significantly enhanced the regeneration of osteochondral defects in rabbits through the complete formation of hyaline cartilage and subchondral bone tissue compared to HCF hydrogels with SDMSCs or growth factors alone.[24]

Thus, the purpose of this study was to evaluate the safety of HCF hydrogel containing autologous human SDMSCs, TGF-β1, and BMP-4 after implantation to articular cartilage defect in patients with osteoarthritis. At this moment, we could not find any article or clinical trial in the literature and specialized databases on the safety and efficacy of injectable hydrogel with encapsulated autologous human SDMSCs and chondroinductve factors in articular cartilage defect repair. Therefore, in this study, we present the first evidence of clinical safety of implantation of HCF hydrogel, containing SDMSCs and growth factors (TGF-β1 and BMP-4), into articular cartilage defect of OA patients.

Materials and Methods

A single-center, prospective observational study was conducted between January 2022 and August 2022 at the National Scientific Center of Traumatology and Orthopedics (Nur-Sultan, Kazakhstan).

The study was conducted in accordance with the ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). The study protocol was reviewed and approved by the Institutional Review Board of the National Center for Biotechnology (№ 00013497) and the Local Ethics Committee of the National Scientific Center of Traumatology and Orthopedics (Protocol №3, 25.12.2021). Written informed consent was obtained from all participants.

Evaluation of Patient Suitability for Study Participation

We included patients with ≥1 chondral/osteochondral lesions of the distal femur as identified by magnetic resonance imaging (MRI) analysis (Figure 1) and/or previous arthroscopies.

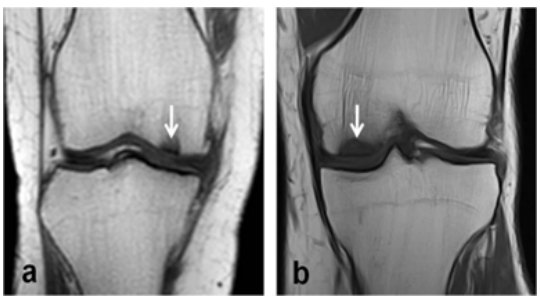

Figure 1. *MRI images of two patients with Grade 2 osteoarthritis. Preoperative MRI imaging revealed a chondral defect (arrow) on the medial femoral condyle (**a** and **b**).*

Inclusion criteria were (1) the age range of 25-65 years, (2) a symptomatic knee joint cartilage defect that was International Cartilage Repair Society (ICRS) Grade III or more under arthroscopy that was recalcitrant to more than 6 months of conservative care, and (3) size of less than 10 cm^2 for a single lesion or 15 cm^2 for multiple lesions with a relatively intact neighboring cartilage (ICRS Grades I and II).

Exclusion criteria were (1) those with more than 5° of varus or valgus deformity in the hip–knee–ankle angle, (2) the presence of instability in the affected joint, including patellofemoral instability due to any coexisting ligament problem, (3) inflammatory arthritis, synovitis, patellofemoral instability, drug and alcohol abuse, and psychological problems.

Isolation and culture of human SDMSCs

Synovial membranes were harvested aseptically from the knee joint of the patients under epidural anesthesia. Isolated synovial membranes were rinsed twice with Dulbecco's phosphate-buffered saline (DPBS) supplemented with 1% penicillin/streptomycin (Gibco, USA), minced into 1-2mm^2 pieces, and digested with 0.25% type II collagenase (Gibco, USA) at 37°C for 4 hours. The cells were washed twice by DPBS and resuspended in StemPro® MSC SFM XenoFree medium (Gibco, USA) supplemented with 1% penicillin/streptomycin. The cells were plated in CELLstart™ CTS™ (Gibco, USA) pre-coated T75 tissue culture flask (Corning, USA) and cultured in StemPro® MSC SFM Xeno Free medium at 37°C and 5% CO_2. Nonadherent cells were removed by day 2, and the adherent cells were propagated before they reached 80-90% confluence. The cells were harvested with TrypLE Express (Gibco, USA) and split in a ratio of 1:3. The culture medium was changed every 2 days. Cell viability was evaluated via trypan blue exclusion with automated cell counter BioRad TC20 (Biorad, USA). The cell culture was periodically tested for contamination with bacteria, yeast, and fungi using the Cell Culture Contamination Detection Kit (Thermo Fisher Scientific, USA).

Immunocytochemistry

Human SDMSCs were grown on a 4-well chambered cell culture slide (BD Biosciences, USA). After fixation with 4% paraformaldehyde and washing with PBS, the cells were incubated with primary antibodies against CD45 (1:100 dilution; ab40763), CD73 (1:100 dilution; ab40763), CD90 (1:100 dilution; ab226133), and CD105 (1:100 dilution; ab231774) (all from Abcam, UK) in PBS/0.1% Tween 20 and 10% normal goat serum (Abcam, UK) overnight at 4°C. The cells were washed with PBS and incubated with goat anti-rabbit IgG Alexa Fluor 488 (1:1000 dilution; A-11008) (Thermo Fisher Scientific, USA) in PBS for 45 min at room temperature. After washing in PBS, the cells were mounted in antifade reagent with DAPI (Thermo Fisher Scientific, USA) for 1 min and visualized with an inverted fluorescence microscope Axio Scope A1 (Carl Zeiss, Germany).

Detection of mycoplasma and bacterial endotoxins in cell culture

The presence of mycoplasma in human SDMSC cultures was detected with the PCR Mycoplasma Test Kit (Applichem, Germany) according to the manufacturer's instructions. The detection of gram-negative bacterial endotoxins in human SDMSC suspension was performed with Pierce™ LAL Chromogenic Endotoxin Quantitation Kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

Synthesis of heparin-conjugated fibrinogen

Heparin-conjugated fibrinogen was prepared according to a previously described protocol.[25] Briefly, 100 mg of low-molecular-weight heparin (LMWH) (Abcam, UK) was dissolved in 100 mL of 0.05M 2-morpholinoethanesulfonic acid monohydrate. In order to activate the -COOH groups of LMWH, 0.04 mM 1-hydroxy-2,5-pyrrolidinedione and 0.08 mM N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride were added and incubated at 4°C for 12 hours. The solution of activated LMWH was shaken vigorously, precipitated with an excess volume of anhydrous acetone, and lyophilized for 24 hours. Subsequently, 100 mg of human plasminogen-free fibrinogen (Sigma, USA) was dissolved in 20 mL phosphate-buffered saline (pH 7.4) at 4°C. This solution subsequently reacted with 60mg of lyophilized LMWH for 3 hours at 4°C. Following precipitation and lyophilization in similar conditions, a powder of heparin-conjugated fibrinogen was dissolved in DPBS. To remove residual LMWH, heparin-conjugated fibrinogen was dialyzed with a dialysis sack (12,000-14,000 Da) at 4°C for 24 hours and lyophilized for 48 hours to produce fibrinogen conjugated to purified heparin. Lyophilized heparin-conjugated fibrinogen was sterilized with 15 kGy dose gamma radiation using the ILU-10 irradiation unit (Park of Nuclear Technologies, Kurchatov, Kazakhstan).

Preparation of HCF hydrogel with human SDMSCs and growth factors

To prepare HCF hydrogel with human SDMSCs and growth factors, the components of fibrin sealant (TISSEEL lyo, Baxter AG, Austria) have been used. For the preparation of the component (1), 91 mg of human fibrinogen, 20mg of heparin-conjugated fibrinogen, 1μg of human recombinant TGF-β1, and 1μg of BMP-4 (Abcam, UK) were dissolved with 1ml of aprotinin solution at 37°C for 45 min using a sterile water bath. After dissolving the fibrin solution, 2×10^7 of autologous human SDMSCs were added and mixed at magnetic stir for 3 min. For the preparation of component (2), 500 IU of human thrombin was dissolved in 1ml of 40μM calcium chloride solution at 37°C for 45 min using a sterile water bath. The reconstituted components (1) and (2) were placed in two single-use syringes, inserted into the DUPLOJECT two-syringe clip, and connected this assembly to the DUPLOTIP dual lumen cannula 20G×10 cm (Baxter, USA). Both syringes were filled with equal volumes.

Surgical procedures

All surgical procedures were conducted under epidural anesthesia after patient informed consent. At the initial stage of the first procedure, arthroscopy of the patient's knee joint was performed to measure the positions, sizes, and depths of cartilage defects, as well as to identify abnormalities of the menisci and ligaments. Then, a synovial membrane biopsy was harvested and sent to the stem cell laboratory (National Center for Biotechnology) for the isolation and expansion of autologous SDMSCs. After 4-5 weeks, a suspension of cultured SDMSCs (2×10^7 cells/vial) with cell viability of more than 90% was delivered for a second surgery.

In the second stage, a mini-arthrotomy of the knee joint was performed on the medial or lateral part of the patella along the cartilage defect. Before implantation of the hydrogel, remnant articular cartilage tissue, fibrotic tissue, and sclerotic bone were debrided and removed from the edges of the cartilage defect (Figure 2).

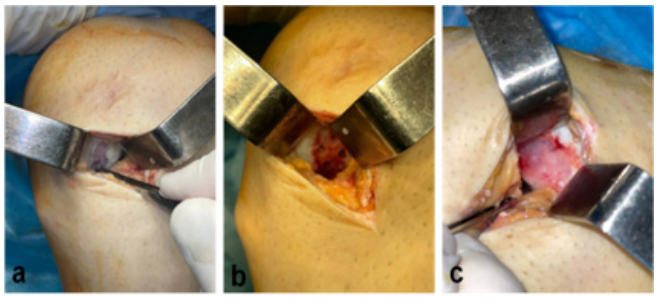

Figure 2. *Surgical procedure of HCF hydrogel implantation into the cartilage defect.* ***(a)*** *Preparation of cartilage defect;* ***(b)*** *Microperforation of subchondral bone in the defect site;* ***(c)*** *Gelated HCF hydrogel after implantation.*

After several rinsing of the defect with sterile saline, several microperforations 5 mm deep and 2.5 mm in diameter were made. After stopping bleeding from the holes using a gauze swab soaked in a solution of epinephrine, the HCF hydrogel containing synovial MSCs and growth factors (TGF-β1 and BMP-4) was implanted using DUPLOJECT two-syringe device connected with the DUPLOTIP dual lumen cannula.

Postoperative rehabilitation

Postoperative rehabilitation was performed individually for each patient, depending on the location and size of the defect, the level of physical activity, and the postoperative progress. Patients should walk with crutches for 6 weeks and not lift heavy objects. Patients should perform isometric hamstring and quadriceps contraction exercises for 6 weeks.

Clinical and radiological evaluation of the knee joint

The knee conditions of the patients before and after surgery were assessed with VAS, WOMAC, and KOOS. Before surgery, MRI was performed on all patients using a 1.5-Tesla MRI system.

Statistical analysis was performed using statistical software package SPSS version 22.0 (SPSS Inc, Chicago, IL). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. The Wilcoxon criterion was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables are performed using chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Out of the 330 screened patients with articular cartilage defects, 15 patients (66.7% women and 33.3% men) with a mean age of 44.2±18.0 years were enrolled in the study and received implantation of HCF hydrogel containing autologous SDMSCs and growth factors (TGF-β1 and BMP-4) (Figure 3). Of the study participants, 4(26.67%) patients had Kellgren-Lawrence Grade I, while 11(73.33%) had Grade II (Figure 1). OA of the right and left knee was diagnosed in 8(53.33%) and 7(46,67%) patients, respectively. The VAS median value at the damaged knee was 59 mm (range of 43–87 mm). The mean lesion size was 4.9±2.0 cm on MRI. All treated defects were on the femur, mostly on the medial femoral condyle - 11(73.33%), lateral femoral condyle - 3(20%), and trochlea - 1(6.67%) (Table 1).

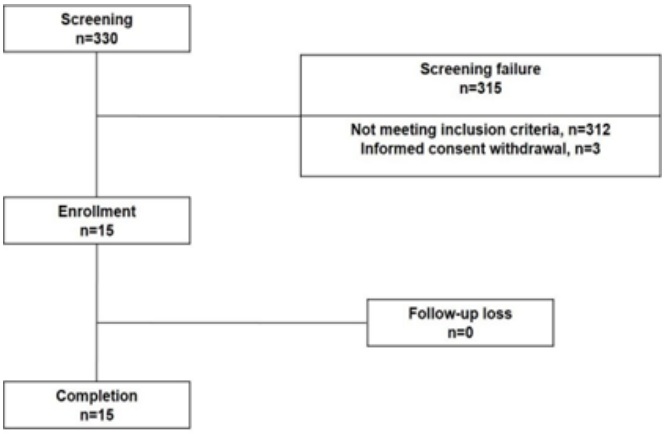

Figure 3. *The study scheme.*

Table 1.

Demographics and baseline characteristics of study participants

Experimental group (n=15)		
Age, years		44±18
Sex	Male	6 (34%)
	Female	9 (66%)
Body mass index, kg/m^2		29.38±3.9
Diagnosis	Osteoarthritis, K-L grade	
	1	4 (26.67%)
	2	11(73.33%)
Knee	Right	8 (53.33%)
	Left	7 (46.67%)
Lesion size, cm^2		4.9±2.0
Location	Medial femoral condyle	11 (73.33%)
	Lateral femoral condyle	3 (20%)
	Trochlea	1 (6.67%)
Compartment	Unicompartmental osteoarthritis	9 (60%)
	Multicompartmental osteoarthritis	6 (40%)
Concomitant procedures	Meniscectomy	13 (86.67%)
	Other procedures	6 (40%)

Evaluation of clinical safety

The safety assessment of hydrogel was based on the absence of infections, inflammation, adhesion, loose body, and tumor formation in the knee joints. Joint pain, synovitis, and edema were observed at an early date after surgery, and all symptoms had completely disappeared at Week 8 after surgery. In addition to assessing the condition of the joints, patients were monitored to determine undesirable side effects after the hydrogel implantation. The results showed no severe adverse events (SAEs) for 8 weeks after the implantation (Table 2).

Table 2.

Adverse events after hydrogel implantation

Adverse events	Before surgery	After surgery (24 h)	After surgery (8 weeks)
Joint pain	9 (60%)	5 (33.3%)	0
Joint edema	3 (20%)	2 (13.3%)	0
Synovitis	3 (20%)	2 (13.3%)	0
Joint contracture	0	0	0
Surgical wound infection	0	0	0

Furthermore, no post-operative infections were detected. There were no significant changes in body temperature and blood pressure before and after hydrogel implantation. There were no adverse events according to the criteria of the World Health Organization after surgery and for the 8-week follow-up. No significant differences were observed before and after implantation regarding overall or specific treatment-emergent adverse events in the initial 24 hours of a clinical trial or the 8-week follow-up (Table 2). No participant was withdrawn from the study because of adverse events. The pain at the incision site was the only severe adverse event considered by the investigator as "probably related" to treatment (due to mini-arthrotomy). In the 8-week follow-up, pain decreased. None of the severe adverse events were considered treatment-related by the investigators. No inflammation reactions were observed in any of the 15 participants treated with HCF hydrogel, according to the blood test.

There was no significant abnormal finding in the hematological parameters during this study (Table 3).

Clinical outcomes of the patients after 6-month follow-up were evaluated with VAS, WOMAC, and KOOS. VAS score and WOMAC-A pain score significantly decreased at all time points compared to baseline (P<0.001 in all cases) (Table 4). Knee pain, other symptoms, and QOL were measured by the corresponding KOOS subscores and showed statistically significant improvement compared to baseline at all time points (P<0.001). The subjective assessment of ADL measured by the ADL KOOS subscore also showed significant enhancement compared to the baseline at all time points.

To evaluate articular cartilage repair, we used the MOCART score. In total, 15 patients underwent MRI before and 6 months after implantation (Figure 4). A total of 11(73.3%) patients showed complete graft integration into the border zone, one had a visible demarcating border, and 3 patients still had visible defects. Intact surface tissue appeared in 11(73.3%) patients, whereas 4(26.7%) patients still had a damaged surface.

Table 3.

Hematological tests at baseline, 24 hours, and Week 8 after HCF hydrogel implantation.

Hematological parameter	Baseline	24 hours	Week 8	Changes (between baseline and 24 h)	Changes (between 24 h and Week 8)	Changes (between baseline and Week 8)	P-value
WBC, $\times 10^9$/L	6.47 ± 2.18	6.76 ± 2.02	6.78 ± 1.45	0.29 ± 1.78	0.024 ± 1.86	0.314 ± 1.96	0.5632
NEU, %	51.3 ± 5.2	58.6 ± 4.2	53.2 ± 4.4	7.3 ± 3.2	-5.4 ± 4.2	1.9 ± 4.1	0.2346
EOS, %	2.5 ± 1.2	2.2 ± 1.6	3.6 ± 1.1	-0.3 ± 1.1	1.4 ± 1.3	1.1 ± 1.3	0.7892
BAS, %	0.4 ± 0.3	0.9 ± 0.2	0.5 ± 0.4	0.5 ± 0.3	-0.4 ± 0.2	0.1 ± 0.3	0.5223
LYM, %	34.4 ± 9.2	30.6 ± 8.3	28.1 ± 7.4	-3.8 ± 6.8	-2.5 ± 7.1	-6.3 ± 7.2	0.2754
MON, %	5.5 ± 3.2	4.3 ± 2.6	4.9 ± 3.3	-1.2 ± 1.1	0.6 ± 0.8	-0.6 ± 0.9	0.3879
RBC, $\times 10^{12}$/L	4.68 ± 0.54	4.74 ± 0.61	4.65 ± 0.75	0.06 ± 0.66	-0.09 ± 0.71	-0.03 ± 0.72	0.4916
Platelet, $\times 10^9$/L	246 ± 46.79	236 ± 42.53	255 ± 50.71	-10 ± 5.54	19 ± 4.33	9.2 ± 4.12	0.3167
ESR, mm/hr	21.2 ± 12.2	23.7 ± 10.3	14.5 ± 8.4	2.5 ± 3.12	-9.2 ± 3.43	-6.7 ± 3.11	0.1257
Total protein, g/dL	6.67 ± 0.28	6.55 ± 0.4	6.34 ± 0.31	-0.12 ± 0.34	-0.21 ± 0.38	-0.33 ± 0.42	0.4021
Total bilirubin, mg/dL	0.65 ± 0.17	0.72 ± 0.13	0.63 ± 0.14	0.07 ± 0.23	-0.09 ± 0.16	-0.02 ± 0.15	0.3219
AST, IU/L	20.2 ± 9.1	21.6 ± 8.3	18.5 ± 7.9	1.4 ± 0.2	-3.1 ± 0.3	-1.7 ± 0.2	0.5223
ALT, IU/L	23.4 ± 10.2	20.2 ± 9.7	19.2 ± 8.8	-3.2 ± 9.1	-1 ± 8.2	-4.2 ± 9.3	0.4034
Total cholesterol, mg/dL	177 ± 30.4	175 ± 35.5	168 ± 20.7	-2 ± 24.8	-7 ± 22.3	-9.1 ± 25.1	0.0854

Abbreviations: *ALT, alanine transaminase; AST, aspartate transaminase; ESR, erythrocyte sedimentation rate; WBC, white blood cell; RBC, red blood cell; NEU, neutrophils; EOS, eosinophils; BAS, basophils; LYM, lymphocytes; MON, monocytes.*

Table 4.

Analysis of the changes in VAS, WOMAC, and KOOS scores before and6 months after HCF hydrogel implantation.

Parameter	Baseline (n=15)		Month 1 (n=15)		Month 6 (n=15)	
	Mean ± SD	(Range)	Mean ± SD	(Range)	Mean ± SD	(Range)
VAS-knee pain	67.54 ± 13.73	(45–84)	22.81 ± 18.21	(40–85)	14.74 ± 17.20	(0–80)
P-value			<0.001		<0.001	
WOMAC-A-knee pain	28.98 ± 14.72	(6–56)	13.63 ± 12.44	(0–37)	7.10 ± 10.12	(0–33)
P-value			<0.001		<0.001	
WOMAC-C–knee function	23.58 ± 15.95	(0–52.94)	9.29 ± 10.33	(0–35.29)	6.45 ± 12.13	(0–47.06
P-value			<0.001		<0.001	
KOOS–Knee pain	70.07 ± 13.24	(41.67–88.89)	85.66 ± 11.89	(55.56–100)	90.28 ± 10.34	(63.89–100)
P-value			<0.001		<0.001	
KOOS–Symptoms	60.65 ± 23.79	(5–95)	78.71 ± 17.89	(30–100)	83.28 ± 14.84	(40–100)
P-value			<0.001		<0.001	
KOOS–Activity of daily living	79.93 ± 14.38	(48.53–100)	92.17 ± 9.75	(64.71–100)	93.97 ± 9.19	(63.24–100)
P-value			<0.001		<0.001	
KOOS–Quality of life	45.16 ± 18.52	(6.25–93.75	61.09 ± 27.29	0–100)	68.27 ± 23.45	(0–100)
P-value			<0.001		<0.001	

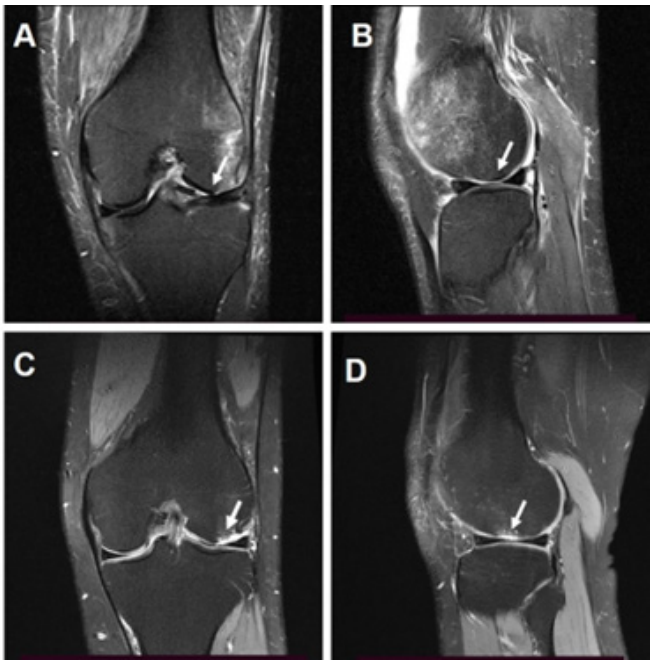

Figure 4. *MRI images of the medial femoral condyle. (A-B) Images of cartilage lesion on medial femoral condyle before HCF hydrogel implantation; (C-D) Images captured after 6-month follow-up showed regeneration of articular cartilage. The white arrow indicates a cartilage lesion.*

The structure of the repaired tissue was homogenous in 12(80%) patients. An isointense signal was seen in 11(73.3%) patients, and 4(26.7%) patients had a moderately hyperintense signal; a markedly hyperintense signal was not observed. Regarding subchondral bone repair, the subchondral lamina was intact in 9(60%) patients, and all patients had intact subchondral bone. Finally, no patients exhibited adhesion, and 12(80%) patients showed no evident effusion.

Discussion

We tested HCF hydrogel as a new drug for the treatment of articulate cartilage defects in small-group OA patients to evaluate its safety and identify side effects. In the present study, an injectable HCF hydrogel for delivery of autologous human SDMSCs, TGF-β1, and BMP-4 was fabricated from FDA–approved materials: LMWH, fibrinogen, thrombin, and aprotinin. Implantation of HCF hydrogel into articular cartilage defect was performed with a mini-arthrotomy approach using DUPLOJECT two-syringe device connected with the DUPLOTIP dual lumen cannula. Our early clinical study demonstrated that implantation of HCF hydrogel with autologous human SDMSCs, TGF-β1 and BMP-4 resulted in a favorable safety profile for patients with OA. No significant severe adverse events or undesirable side effects such as joint edema, inflammation reactions, elevated body temperature, and wound infections after HCF hydrogel implantation were observed during this study. In addition, our laboratory tests showed no significant abnormal changes in the hematological parameters of the patients during post-surgical follow-up. Only the pain at the incision site was reported as a minor adverse event, but the pain gradually disappeared in 8 weeks after surgery.

To our knowledge, this is the first report of a clinical study on the safety of the implantation of HCF hydrogel with encapsulated human SDMSCs and chondroinductive factors into articular cartilage defect in OA patients.

In the literature, only two previous clinical studies used autologous MSCs loaded in fibrin glue for the treatment of cartilage defects in the human knee. A pilot study of 5 patients with full-thickness cartilage defects revealed that implantation of autologous bone marrow-derived MSCs loaded on platelet-rich fibrin glue significantly improved functional knee scores and MRI findings at 6 and 12 months postoperatively.[26] Kim and colleagues showed that fibrin glue is an effective and safe injectable scaffold in the implantation of adipose-derived stem cells for the treatment of articular cartilage defects in OA patients.[27]

In our study, we used autologous SDMSCs because they have a number of advantages in comparison to MSCs isolated from other sources: 1) SDMSCs can be easily isolated in a sufficient amount from a small piece of the synovial membrane, 2) they do not lose their phenotypic properties during cultivation, 3) the functional activity of SDMSCs remains at a high level regardless of the person's age.[11] Moreover, SDMSCs are tissue-resident stem cells that have a higher capacity to differentiate into chondrocytes than MSCs from bone marrow or adipose tissue. To obtain safe cell product, SDMSCs were propagated in serum-free and xeno-free culture medium StemPro® MSC and examined accurately for the presence of bacteria, fungi, yeast, mycoplasma, and bacterial endotoxins in cell culture. During our study, no SDMSCs containing any abovementioned contaminants were observed.

A number of studies have shown that the application of recombinant growth factors alone or in combination with MSCs can stimulate cell proliferation, extracellular matrix (ECM) formation, and tissue regeneration. However, despite the therapeutic efficacy of recombinant growth factors, the clinical application is still limited due to a short half-life and poor biological activity.[28] Large doses of TGFs and BMPs cause adverse effects, including immune response and abnormal growth of cartilage and bone tissue.[29] Therefore, to overcome the aforementioned limitations, many researchers have developed drug delivery systems based on natural and/or synthetic scaffolds for the sustained release of growth factors in a target site. Among the scaffolds for growth factors and cell delivery, injectable fibrin hydrogels demonstrated great potential in cartilage and bone tissue engineering. The fibrin hydrogel application is supported by its high-water content, biocompatibility, biodegradability, nontoxicity, porous framework, structural similarity to the ECM, and ability to match irregular defects.[30,31] Conjugating heparin to fibrinogen provides biomaterials for the controlled release of heparin-binding proteins such as TGFs and BMPs. The heparin-conjugated hydrogel can protect growth factors from proteolysis and prolong the retention of their biological activity in vivo.[29] In addition, it has been shown that heparin can modulate the biological activity of the TGF-β1, which plays a significant role in cell migration, proliferation, cartilage differentiation, and synthesis of cartilage-specific ECM, which is also involved in the suppression of immune response.[32,33]

Therefore, in our study, we used HCF hydrogel as a scaffold for the delivery of human recombinant TGF-β1 and BMP-4 in combination with SDMSCs because we supposed that HCF hydrogel could better stimulate the regeneration of articular cartilage defect in OA patients in comparison with MSC implantation in fibrin gel. Our first-in-human clinical study showed that implantation of SDMSCs with human recombinant TGF-β1 and BMP-4 in HCF hydrogel did not lead to significant adverse events and changes in hematological profiles of OA patients. Thus, we consider HCF hydrogel as a safe injectable scaffold for the delivery MSCs and chondroinductive factors for the treatment of cartilage defects.

Moreover, the analysis of clinical outcomes showed that HCF hydrogel is effective for the treatment of articular cartilage defects in OA patients between 25 and 65 years. As measured by the KOOS scores, the main clinical improvement was presented in a significant increase in knee function, which was observed 6 months post-treatment. WOMAC-C score showed a similar pattern confirmed by clinical changes during post-surgery time. The results of the present study showed that hydrogel implantation is effective in pain reduction by providing lasting therapeutic effects as also shown by changes in VAS, WOMAC-A, and KOOS scores. Function and symptom improvements led to a significant increase in ADL and QOL in 30 days. Thus, HCF hydrogel containing autologous SDMSCs and chondroinductive growth factors promotes rapid and lasting therapeutic effects in OA patients with Kellgren-Lawrence Grade I-II.

This study has some limitations. First, the number of OA patients in an experimental group was relatively small. Second, the follow-up period after implantation of HCF hydrogel with SDMSCs and growth factors was short for the examination of long-term safety. For a complete assessment of HCF hydrogel as a carrier of SDMSCs and growth factors in articular cartilage defects of OA patients, a prospective study with a larger number of patients and a long-term follow-up period is required. Currently, we have enrolled 40 subjects and carried out a 2-year clinical study to evaluate the long-term safety and therapeutic efficacy of HCF hydrogel.

Conclusion

The results of this first-in-human clinical study showed that the application of HCF hydrogel with autologous SDMSCs, TGF-β1, and BMP-4 appears to be safe and showed no serious adverse events in OA patients. The implantation of HCF hydrogel was found to be a promising method for the effective repair of cartilage defects in femoral condyles, with the effect lasting for 6 months after the HCF hydrogel implantation for the vast majority of the patients. The HCF hydrogel-based grafting technique appears to be a viable option for condylar lesions and may delay or prevent the need for arthroplasty. However, further clinical study is required to evaluate the long-term safety and therapeutic efficacy of HCF hydrogel using a larger number of subjects.

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Competing Interests

The authors declare that they have no competing interests

References

1. Hiligsmann M, Reginster JY. The economic weight of osteoarthritis in Europe. J Medicographia. 2013;35:197–202.
2. Klug A, Gramlich Y, Rudert M, Drees P, Hoffmann R, Weißenberger M, Kutzner KP. The projected volume of primary and revision total knee arthroplasty will place an immense burden on future health care systems over the next 30 years. Knee Surg Sports Traumatol Arthrosc. 2021 Oct;29(10):3287-3298. doi: 10.1007/s00167-020-06154-7.
3. Safiri S, Kolahi AA, Smith E, Hill C, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017. Ann Rheum Dis. 2020 Jun;79(6):819-828. doi: 10.1136/annrheumdis-2019-216515.
4. Evenbratt H, Andreasson L, Bicknell V, Brittberg M, Mobini R, Simonsson S. Insights into the present and future of cartilage regeneration and joint repair. Cell Regen. 2022 Feb 2;11(1):3. doi: 10.1186/s13619-021-00104-5.
5. Steinert AF, Ghivizzani SC, Rethwilm A, Tuan RS, Evans CH, Nöth U. Major biological obstacles for persistent cell-based regeneration of articular cartilage. Arthritis Res Ther. 2007;9(3):213. doi: 10.1186/ar2195.
6. Huang K, Li Q, Li Y, Yao Z, Luo D, Rao P, Xiao J. Cartilage Tissue Regeneration: The Roles of Cells, Stimulating Factors and Scaffolds. Curr Stem Cell Res Ther. 2018;13(7):547-567. doi: 10.2174/1574888X12666170608080722.
7. Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noël D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. Trends Biotechnol. 2009 May;27(5):307-14. doi: 10.1016/j.tibtech.2009.02.005..
8. Huselstein C, Li Y, He X. Mesenchymal stem cells for cartilage engineering. Biomed Mater Eng. 2012;22(1-3):69-80. doi: 10.3233/BME-2012-0691.
9. Salgado AJ, Oliveira JT, Pedro AJ, Reis RL. Adult stem cells in bone and cartilage tissue engineering. Curr Stem Cell Res Ther. 2006 Sep;1(3):345-64. doi: 10.2174/157488806778226803.
10. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. Cell Transplant. 2011;20(1):5-14. doi: 10.3727/096368910X.
11. Fan J, Varshney RR, Ren L, Cai D, Wang DA. Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration. Tissue Eng Part B Rev. 2009 Mar;15(1):75-86. doi: 10.1089/ten.teb.2008.0586.
12. Sasaki A, Mizuno M, Ozeki N, Katano H, Otabe K, Tsuji K, Koga H, Mochizuki M, Sekiya I. Canine mesenchymal stem cells from synovium have a higher chondrogenic potential than those from infrapatellar fat pad, adipose tissue, and bone marrow. PLoS One. 2018 Aug 23;13(8):e0202922. doi: 10.1371/journal.pone.0202922.
13. Jones BA, Pei M. Synovium-derived stem cells: a tissue-specific stem cell for cartilage engineering and regeneration. Tissue Eng Part B Rev. 2012 Aug;18(4):301-11. doi: 10.1089/ten.TEB.2012.0002.
14. Koga H, Muneta T, Ju YJ, Nagase T, Nimura A, Mochizuki T, Ichinose S, von der Mark K, Sekiya I. Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. Stem Cells. 2007 Mar;25(3):689-96. doi: 10.1634/stemcells.2006-0281.
15. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum. 2005 Aug;52(8):2521-9. doi: 10.1002/art.21212.
16. Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res. 2008 Aug;333(2):207-15. doi: 10.1007/s00441-008-0633-5.
17. Magne D, Vinatier C, Julien M, Weiss P, Guicheux J. Mesenchymal stem cell therapy to rebuild cartilage. Trends Mol Med. 2005 Nov;11(11):519-26. doi: 10.1016/j.molmed.2005.09.002.
18. Miljkovic ND, Cooper GM, Marra KG. Chondrogenesis, bone morphogenetic protein-4 and mesenchymal stem cells. Osteoarthritis Cartilage. 2008 Oct;16(10):1121-30. doi: 10.1016/j.joca.2008.03.003.
19. Wagenbrenner M, Mayer-Wagner S, Rudert M, Holzapfel BM, Weissenberger M. Combinations of Hydrogels and Mesenchymal Stromal Cells (MSCs) for Cartilage Tissue Engineering-A Review of the Literature. Gels. 2021 Nov 16;7(4):217. doi: 10.3390/gels7040217.
20. Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Pawde AM, Kumar GS, Sharma GT. Mesenchymal stem cells with IGF-1 and TGF- β1 in laminin gel for osteochondral defects in rabbits. Biomed Pharmacother. 2017 Sep;93:1165-1174. doi: 10.1016/j.biopha.2017.07.032.
21. Huang H, Hu X, Zhang X, Duan X, Zhang J, Fu X, Dai L, Yuan L, Zhou C, Ao Y. Codelivery of Synovium-Derived Mesenchymal Stem Cells and TGF-β by a Hybrid Scaffold for Cartilage Regeneration. ACS Biomater Sci Eng. 2019 Feb 11;5(2):805-816. doi: 10.1021/acsbiomaterials.8b00483.
22. Vayas R, Reyes R, Arnau MR, Évora C, Delgado A. Injectable Scaffold for Bone Marrow Stem Cells and Bone Morphogenetic Protein-2 to Repair Cartilage. Cartilage. 2021 Jul;12(3):293-306. doi: 10.1177/1947603519841682.
23. Ogay VB, Isabekova AS, Sarsenova MA, Ramankulov EM. Patent of the Republic of Kazakhstan. A method for obtaining an injectable biocomposite hydrogel to stimulate the regeneration of bone and cartilage tissue. 2019;33784:29.

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24. Sarsenova M, Raymagambetov Y, Issabekova A, Karzhauov M, Kudaibergen G, Akhmetkarimova Zh, Batpen A, Ramankulov Y, Ogay V. Regeneration of Osteochondral Defects by Combined Delivery of Synovium-Derived Mesenchymal Stem Cells, TGF-β1 and BMP-4 in Heparin-Conjugated Fibrin Hydrogel. Polymers. 2022 (under review).
25. Yang HS, La WG, Bhang SH, Jeon JY, Lee JH, Kim BS. Heparin-conjugated fibrin as an injectable system for sustained delivery of bone morphogenetic protein-2. Tissue Eng Part A. 2010 Apr;16(4):1225-33. doi: 10.1089/ten.TEA.2009.0390.
26. Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, et al. The Clinical Use of Human Culture-Expanded Autologous Bone Marrow Mesenchymal Stem Cells Transplanted on Platelet-Rich Fibrin Glue in the Treatment of Articular Cartilage Defects: A Pilot Study and Preliminary Results. Cartilage. 2010 Oct;1(4):253-261. doi: 10.1177/1947603510366027.
27. Kim YS, Choi YJ, Suh DS, Heo DB, Kim YI, Ryu JS, Koh YG. Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? Am J Sports Med. 2015 Jan;43(1):176-85. doi: 10.1177/0363546514554190.
28. Ren X, Zhao M, Lash B, Martino MM, Julier Z. Growth Factor Engineering Strategies for Regenerative Medicine Applications. Front Bioeng Biotechnol. 2020 Jan 21;7:469. doi: 10.3389/fbioe.2019.00469.
29. Joung YK, Bae JW, Park KD. Controlled release of heparin-binding growth factors using heparin-containing particulate systems for tissue regeneration. Expert Opin Drug Deliv. 2008 Nov;5(11):1173-84. doi: 10.1517/17425240802431811.
30. Wei W, Ma Y, Yao X, Zhou W, Wang X, Li C, Lin J, He Q, Leptihn S, Ouyang H. Advanced hydrogels for the repair of cartilage defects and regeneration. Bioact Mater. 2020 Oct 10;6(4):998-1011. doi: 10.1016/j.bioactmat.2020.09.030.
31. Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X, Li S, Deng Y, He N. Injectable hydrogels for cartilage and bone tissue engineering. Bone Res. 2017 May 30;5:17014. doi: 10.1038/boneres.2017.14.
32. Palladino MA, Morris RE, Starnes HF, Levinson AD. The transforming growth factor-betas. A new family of immunoregulatory molecules. Ann N Y Acad Sci. 1990;593:181-7. doi: 10.1111/j.1749-6632.1990.tb16110.x.
33. McCaffrey TA, Falcone DJ, Du B. Transforming growth factor-beta 1 is a heparin-binding protein: identification of putative heparin-binding regions and isolation of heparins with varying affinity for TGF-beta 1. J Cell Physiol. 1992 Aug;152(2):430-40. doi: 10.1002/jcp.1041520226.