Journal of Cartilage & Joint Preservation® 4 (2024) 100170

Contents lists available at ScienceDirect

Journal of Cartilage & Joint Preservation®

journal homepage: www.elsevier.com/locate/jcjp

Original Research

Single-stage autologous chondrocyte coimplantation on a hyaluronan scaffold for the treatment of knee cartilage lesions: a case series of 16 patients with clinical outcomes up to 5 years

Emmanouil T. Papakostas[a,b,*], Efthymios Papasoulis[b], Willem Cornelis de Jong[c], Aristotelis S. Sideridis[b], Argiris Karavelis[b], Konstantinos Epameinontidis[d] and Ioannis P. Terzidis[b]

[a] Aspetar Orthopaedic and Sports Medicine Hospital, Surgery Dpt, Doha, Qatar
[b] Thessaloniki Minimally Invasive Surgery (The-MIS) Orthopaedic Center, St. Luke's Hospital, Thessaloniki, Greece
[c] Cartilage Repair Systems, LLC, New York, NY, USA
[d] Aspetar Orthopaedic and Sports Medicine Hospital, Rehab Dpt, Doha, Qatar

ARTICLE INFO

Keywords:
Chondrocyte coimplantation
Hyaluronan scaffold
Knee cartilage repair
Mononuclear bone marrow cells
One stage
Primary articular chondrocytes

ABSTRACT

Introduction: The direct reimplantation of autologous primary articular chondrocytes in a single-stage procedure for knee cartilage lesions is a novel approach, yet to be extensively reported. This study aimed to evaluate the clinical outcomes and reinterventions over a 5-year period post-surgery.

Methods: A prospective case series involving 16 patients (4 female, 12 male) with single or multiple focal knee cartilage lesions was conducted. The mean age at baseline was 36.8 ($\pm$11.5) years. The mean total lesion size was 4.5 ($\pm$2.3) cm^2 Patients underwent surgery where articular chondrocytes and mononuclear bone marrow cells were isolated, mixed, and seeded onto a hyaluronan-based scaffold within the lesion. A structured physical therapy regimen was followed, and patients were assessed using Knee injury and Osteoarthritis Outcome Score (KOOS) and International Knee Documentation Committee (IKDC) Subjective questionnaires at various intervals. Overall, patient-reported outcomes improved over the first 3 years post-surgery, with slight declines thereafter.

Results: Significant improvements over baseline were noted for various KOOS parameters and IKDC Subjective scores at different follow-up points. Reinterventions were required for two patients, one receiving intra-articular injections with mesenchymal stromal cells and another undergoing knee washout for septic arthritis while a meniscus implant was removed. Patient satisfaction at final follow-up was generally favorable. Despite a significant intraoperative cell isolation time of approximately 1.5 hours, the procedure demonstrated safety and efficacy, with an average of $\pm$0.9 million articular chondrocytes obtained per case.

Conclusions: This study underscores the potential of coimplantation of intraoperatively isolated articular chondrocytes and bone marrow cells on a hyaluronan scaffold as a promising strategy for treating symptomatic knee cartilage lesions.

* Emmanouil T. Papakostas, Aspetar Orthopaedic and Sports Medicine Hospital, Sport City Street, Near Khalifa Stadium, P.O. Box 29222, Doha, Qatar.
Email addresses: emmanouil.papakostas@aspetar.com, epapacostas@gmail.com (E.T. Papakostas).

https://doi.org/10.1016/j.jcjp.2024.100170
Received 20 January 2024; Revised 1 March 2024; Accepted 6 March 2024
Available online 9 March 2024

Introduction

Focal lesions of the articular cartilage of the knee can provoke and propagate joint degeneration and, ultimately, osteoarthritis. Therefore, when focal cartilage lesions become symptomatic, surgical intervention may be elected to deploy cells, such as chondrocytes, in the lesion to grow back a functional repair tissue. One such intervention is autologous chondrocyte implantation (ACI), which is indicated for "larger" chondral lesions in the tibiofemoral and patellofemoral joint, referring to lesion sizes exceeding 4 cm^2[1] or, alternatively, exceeding 2 cm^2.[2] At the center of ACI are the articular chondrocytes—a collective term for the cells found in articular cartilage—which have a specific origin within the development of the synovial joint[3] and appear to be the sole contributors to the mostly postnatally synthesized articular cartilage.[4] Despite being articular-chondrocyte-based, ACI has its drawbacks: it requires 2 surgeries and 2 postoperative recoveries, the in-between laboratory expansion of the chondrocytes is costly and shifts the cells' phenotype, and, incidentally, a chondrocyte culture may fail.

In the last decade and a half, advances have been made in the rapid isolation of chondrocytes from articular cartilage,[5] which allowed for single-surgery treatments using *primary*, that is, nonculture-expanded, autologous articular chondrocytes.[6,7] Although chondrocytes, with or without their pericellular matrix, can be harvested from a cartilage biopsy in the operating room (OR) for direct reuse, the yield may be considered too low for a satisfactory cell density in the lesion. To address this, the primary chondrocytes can be supplemented with a second cell type, such as mononuclear bone marrow cells, which are relatively easy to obtain. The addition of a stromal cell type can function as a partial chondrocyte supplantation, as stromal cells can stimulate the chondrocytes to increase their synthesis of hyaline matrix components.[8–10]

To keep the cells in the lesion in an evenly distributed manner, a 3-dimensional biodegradable cell carrier is necessary, similar to second-generation and third-generation ACI.[11] The carrier provides a temporary scaffold against which a new extracellular matrix can be deposited. Good long-term ($\sim$9 years) clinical results have been obtained with a hyaluronan-based scaffold precultured in vitro with autologous chondrocytes.[12] In patients older than 40 years and in male high-level soccer players, this approach provided promising clinical results as well.[13,14] Two advantages of the hyaluronan-based scaffold used in these studies are: (1) cell seeding can be done *after* positioning of the scaffold into the lesion, so that cell death resulting from preseeded scaffold manipulation is avoided,[15] and (2) the hyaluronan-derived components of the scaffold may be internalized and used by chondrocytes to synthesize new hyaluronan.[16,17]

Two single-surgery autologous chondrocyte coimplantation treatments have been reported in the literature.[6,7] Each has an important limitation: INSTRUCT[7] requires the placement of an osteochondral scaffold, thereby damaging the subchondral bone, and IMPACT[6] (Instant MSC Product Accompanying Autologous Chondron Transplantation) requires the coimplantation of allogeneic mesenchymal stem cells instead of cells of an autologous source. These limitations can be avoided by coimplanting the intraoperatively obtained chondrocytes with autologous mononuclear cells from the patient's own bone marrow and using a chondral scaffold to seed the cell combination in the lesion. We hypothesized that such a strategy is safe and feasible and provides improvement in patient-reported outcomes. To test this hypothesis, 16 patients who presented with symptomatic focal knee cartilage lesions were treated with autologous, intraoperatively isolated articular chondrocytes and mononuclear bone marrow cells. The cell mixture was deployed in the cartilage lesion on a chondral hyaluronan-based scaffold. The patients were followed up for 5 years, and we here report the outcomes.

Materials and methods

Patient cohort

A prospective case series was initiated at the Thessaloniki Minimally Invasive Surgery Orthopaedic Center of the St. Luke's Hospital in Thessaloniki, Greece. The case series included 16 patients, 4 females and 12 males, who underwent surgery between September 2014 and May 2018. The hospital's Ethics Committee approved the study. Inclusion criteria were: single or multiple symptomatic, focal, partial-thickness or full-thickness chondral lesions (grades 2 or 3 of the International Cartilage Regeneration and Joint Preservation Society classification)[18] of the articulating surfaces on the femur or patella, with a size more than 2 cm^2, in patients 14 to 56 years old. Concomitant pathologies that could be addressed simultaneously (stability, malalignment, meniscal repair, or substitution) were not reasons for exclusion. Cases with osteoarthritis (diffuse cartilage pathology, degenerative or rheumatoid), nonfunctional meniscal tissue (more than 50% meniscectomy and/or absence of continuity of hoop fibers), and active inflammatory diseases were excluded. The lesion grade was assessed with magnetic resonance imaging and later confirmed intraoperatively. One patient was treated for focal cartilage lesions in both knees. The mean age at baseline was 36.8 ($\pm$11.5) years. One patient was 14 years old at the time of surgery but had closed epiphyseal growth plates. The mean total lesion area per patient was 4.5 ($\pm$2.3) cm^2 and ranged from 1.8 to 8.8 cm^2. Lesion etiology was trauma in 8 cases and overuse in the other 8 cases. Various concurrent interventions were performed, amongst others partial meniscectomy and reconstruction of the anterior cruciate ligament (Table 1).

Surgical procedure

Surgery was performed with the patient in a supine position under general anesthesia. A thigh-high tourniquet was placed to avoid excessive bleeding. The precise nature, location, and shape of the lesion or lesions were investigated arthroscopically to confirm the suitability of the treatment. Lesions were then carefully debrided, so that their circumference was sturdy, with the lesion wall apparently perpendicular to the subchondral bone and composed of healthy cartilage only. The lesion area was determined with a ruler. The debrided cartilage was collected and supplemented with approximately 0.1 g of healthy-appearing less-load-bearing

cartilage. Bone marrow was aspirated from the ipsilateral or contralateral iliac crest. The volume of aspirate depended on the cartilage lesion area, with more aspirate drawn for larger lesions. Qualified cell technicians processed the collected cartilage pieces and bone marrow aspirate in the OR under sterile laminar airflow to retrieve primary chondrocytes (PCs) from the cartilage and mononuclear bone marrow cells (MNCs) from the aspirate (CartiONE, Cartilage Repair Systems, LLC). The isolated cells were washed, counted, mixed, and resuspended in a small seeding volume in the OR.[19] The amount of MNCs added to the PCs was controlled, so that the final cell mixture suspension provided the surgeon with a total of approximately 9 million cells/cm^2 of lesion. In all 16 cases, the scaffold was a nonwoven single-layer biodegradable hyaluronic acid-based scaffold (HyaloFast, Anika Therapeutics). The surgeon cut the scaffold to shape on the sterile back table and positioned it into the lesion, arthroscopically, if possible, under dry conditions or with CO_2 arthroscopy. In 6 patients (7 knees, all patella cases) the procedure was done through medial parapatellar arthrotomy. Using a low dead-volume syringe, the PC + MNC cell suspension was seeded dropwise into the scaffold to aim for a homogeneous distribution of the cells. The scaffold was then covered with fibrin glue, which was allowed to set before the knee was flexed a few times to confirm that the scaffold remained in place. Care was taken to manipulate the scaffold as little as possible after cell seeding.

Postoperative rehabilitation

Patients followed a structured rehabilitation protocol based on the principles outlined by Mithoefer et al.[20] All patients were given written rehabilitation instructions (home-based or in a physical therapy center of their own choice) from our standard cartilage rehabilitation program (Table 2), and were followed by our rehabilitation team on the regular orthopedic clinical follow-up visits (weeks 2, 4, 6, 8, and months 3, 6, 9, and 12) and advised on the rehabilitation progression in accordance with the protocol and the clinical improvement. If a follow-up appointment was missed, the lead physiotherapist (K.E.) contacted the patient and his/her physio to ensure protocol adherence. The protocol phases were adjusted to the generally accepted healing phases of the healing graft (Table 2). In the initial phase, the primary aims were to protect the graft from excessive loads and shear forces, while allowing for some movement to prevent the detrimental effects of immobilization. In this phase, range of motion and weight-bearing restrictions could apply, and neuromuscular activation exercises were a key component. Restoration of normal gait was a key criterion to proceed to the second phase of rehabilitation. In the second phase, strength training was the main element to restore force production and muscle hypertrophy in a controlled manner. In the third phase, the rate of force development and the restoration of power and explosive strength were the main domains of the protocol for athletic patients, while the nonathletic population focused on functional training that simulated activities of daily living.

Patient-reported outcome measures

Patients completed the Knee injury and Osteoarthritis Outcome Score (KOOS)[21] and the International Knee Documentation Committee (IKDC) Subjective Knee Form[22] questionnaires before surgery and at 3, 6, 12, 24, 36, 48, and 60 months after surgery. At the last follow-up, the patient indicated his/her level of satisfaction with the treatment on a 4-level ordinal scale: dissatisfied, mildly satisfied, much satisfied, very much satisfied.

Statistics

Throughout the Results section, sample means are given with the standard deviation between parentheses. One patient received the treatment under study in both knees (subject 2 in Table 1). This patient reported different scores for the 2 knees. It was therefore decided to define the sample size as the number of knees instead of the number of patients.

To test whether the KOOS and IKDC scores changed over time, classical (not Bayesian) repeated measures analyses of variance (RMANOVA) were done. Mauchly's test was used to test the assumption of sphericity. If the assumption of sphericity was violated, the Greenhouse-Geisser correction of the F-statistic was used. When the KOOS or IKDC score samples were significantly different between follow-up times, between-sample post hoc comparisons were made with Bonferroni adjustment. Error terms for the repeated measures factors were pooled. The RMANOVA was done with JASP software (version 0.18.1, JASP Team, 2023).

Results

Intraoperative cell isolations

The amount of articular cartilage biopsy material collected per patient (debridement plus less-load-bearing cartilage) was 0.72 (±0.29) g, yielding 1.26 (±0.70) million primary chondrocytes (PCs) per gram. The amount of primary articular chondrocytes obtained per patient was 0.924 (±0.658) million. The volume of bone marrow aspirate collected was 20.1 (±3.4) mL, yielding 5.5 (±2.6) million mononuclear bone marrow cells (MNCs) per milliliter. The number of PCs deployed per square centimeter of the lesion was 0.22 (±0.16) million/cm^2 and the percentage of PCs in the cell mixture was 2.5 (±1.6)%. The total areal cell supply (PCs + MNCs) was 9.0 (±2.1) million/cm^2. Variation in the total areal cell supply was introduced because of a more precise estimation of the lesion size only after the PC + MNC cell suspension had been prepared.

Table 1
Cohort demographics.

Subject #	Age at baseline (years)	Sex	Physical activity level	Lesion location(s) with individual lesion area (cm^2) and knee	Total lesion area (cm^2)	Etiology	Time since onset symptoms	Previous intervention(s)	Concurrent intervention(s)
1	45	Male	Sports sometimes	MFC, R	3.8	Trauma	< 1 year	-	20% Resection of MM
2	37	Female	Nonsporting	Patellae (1.4 + 2.4), L + R	3.8	Overuse	> 1 year	-	TTT osteotomy, right knee
3	39	Male	Sports sometimes	Patella, L	3.2	Overuse	> 1 year	MACI in contralateral knee	-
4	43	Male	Sports sometimes	MFC (5.0) + LFC (3.6), R	8.6	Overuse	> 1 year	-	Debridement of trochlear lesion
5	52	Male	Sports frequently/ well-trained	MFC, L	3.4	Trauma	< 1 year	-	Debridement of LFC and trochlear lesions, 25% resection of MM
6	43	Female	Nonsporting	MFC (1.9) + patella (0.5), L	2.4	Overuse	> 1 year	-	20% Resection of MM, resection of IP, arthroscopic debridement of cartilage lesion in the contralateral knee
7	56	Female	Nonsporting	MFC, R	2.9	Overuse	> 1 year	-	Debridement of patellar and trochlear lesions, debridement of both menisci
8	31	Male	High-level competitive sporter	LFC, L	8.4	Trauma	> 2 years	ACL reconstruction, subtotal lateral meniscectomy	Debridement of the trochlear lesion, lateral Actifit placement, removal of loose body
9	20	Male	High-level competitive sporter	LFC, L	1.8	Trauma	2 months	-	Debridement of LTC lesion, debridement of LM, resection of IP
10	30	Male	Sports sometimes	LFC (2.5) + trochlea (6.3), L	8.8	Overuse	> 1 year	-	Resection of LM
11	14	Male	Sports frequently/ well-trained	Patella, L	6.0	Trauma	1 month	-	Reconstruction of MPFL
12	33	Male	Sports sometimes	Trochlea (2.6) + patella (2.4), R	5.0	Trauma	> 1 year	Cartilage debridement, TTT osteotomy	Resection of IP
13	43	Male	Sports sometimes	MFC (1.9) + trochlea (1.4), R	3.3	Overuse	> 1 year	-	ACL Reconstruction, resection of LM
14	47	Female	Nonsporting	MFC (4.3) + LFC (1.0), R	5.3	Trauma	> 1 year	-	Debridement of patellar lesion, resection of IP
15	26	Male	Sports sometimes	MFC, L	2.5	Trauma	1 month	-	ACL reconstruction, 10% resection of MM
16	29	Male	Sports sometimes	Trochlea, L	3.5	Overuse	< 1 year	-	-
mean ± SD	36.8 ± 11.5				4.5 ± 2.3				

Abbreviations: ACL, anterior cruciate ligament; IP, infrapatellar plica; L, left knee; LFC, lateral femoral condyle; LM, lateral meniscus; LTC, lateral tibial condyle; MACI, matrix-assisted autologous chondrocyte implantation; MFC, medial femoral condyle; MM, medial meniscus; MPFL, medial patellofemoral ligament; R, right knee; TTT, tibial-tubercle transfer.

Table 2
Postoperative rehabilitation protocol.

	Tibiofemoral lesions		Patellofemoral lesions	Criteria to progress to next phase
Phase 1: Protection	Contained lesions *Brace* Week 1: locked at 0° Week 2-3: 0-60° Week 4: 0-90°, then remove *ROM* Week 1: passive or active-assisted movements Week 2-3: active-assisted Week 4: active movements *WB status*[*] Week 1: toe-touch WB Week 2: 50% WB Week 3: 75% WB Week 4: FWB as tolerated Cryotherapy and compression Thigh-muscle activation exercises & NMES Gait retraining Hip strengthening exercises Calf strengthening exercises	Uncontained lesions *Brace* Week 1: locked at 0° Week 2-3: 0-30° Week 4-5: 0-60° Week 6: 0-90°, then remove *ROM* Week 1: passive movements Week 2-4: active-assisted Week 5-6: active movements *WB status*[*] Week 1: NWB Week 2: 20% WB Week 3-4: 50% WB Week 5-6: 75% WB Week 7-8: FWB	*Brace* Week 1-2: locked at 0° Week 3: 0-30° Week 4: 0-60° Week 5-6: 0-90° *ROM* Week 1: passive movements Week 2-4: active-assisted Week 5-6: active movements *WB status* Week 1: WB as tolerated FWB as tolerated	• Full passive ROM • Minimal pain on VAS (< 3/10) • Minimal swelling • Good muscle activation observed • Restored gait pattern
Phase 2: Progressive loading and restoring joint function	Strength training drills Low load (30-50% 1 RM)—high repetitions Progress to higher load (70% 1 RM) not earlier than 10 wk Initiate low-load plyometric activity after week 16, if tolerated		Strength training drills Low load (30-50% 1 RM)— high repetitions Avoid OKC with resistance until week 12 Low-load plyometrics not earlier than week 16	• Pain-free ROM • Minimal or absent swelling • Strength test difference < 20% LSI
Phase 3: Restoring normal activity	Increase load to 85% 1 RM—low repetitions Introduce plyometrics (moderate to high). Introduce power drills Explosive strength (for athletes only)		High-load strength training initiated after week 20 Moderate plyometrics after week 24, if tolerated Explosive strength, power, and agility drills as tolerated	To Discharge: • Pain-free • No swelling • Strength test 100% LSI for athletes, 90% for nonathletes • PROMs 100% on pain and symptoms subscales, 80% for nonathletes

Abbreviations: FWB, full weight-bearing; LSI, limb symmetry index; NMES, neuromuscular electrical stimulation; NWB, nonweight-bearing; OKC, open kinetic chain; PROMs, patient-reported outcome measures; RM, repetition maximum; ROM, range of motion; VAS, visual analog scale; WB, weight-bearing; wk, week.

[*] Loading may vary based on lesion size and patient characteristics.

ORtimes

The intraoperative time from the end of cartilage collection to the cell mixture being ready for seeding was 96 (±16) minutes. The surgery time, from first incision to wound closure, was 165 (±29) minutes. (Both time samples: $n = 14$, no records were available for 2 cases). The tourniquet was deflated during the cell isolation procedure and reinflated in preparation for scaffold and cell mixture placement so that the total use of the tourniquet per case was always less than 120 minutes.

Adverse events including reinterventions

A reintervention of the index knee following the treatment under study was performed in 2 subjects. One patient (subject 8) who had a concomitant placement of a partial meniscus implant during the surgery presented with septic arthritis at 3 weeks post-operatively. The partial meniscus implant was removed, and the knee was washed out once (at 3 weeks post surgery) with proper antibiotic therapy for 12 weeks. One other patient (subject 5) was treated with intra-articular injections of mesenchymal stromal cells (MSCs), because of residual pain and disability. The treatment was performed in another center with fat-derived MSCs after cell expansion in 2 stages. The patient informed the lead author verbally about this MSC treatment. The treatment was performed 8 months after the surgical intervention. Apart from mild swelling during the immediate postoperative period, no other adverse events were reported during the follow-up period by any patient.

Patient-reported outcome measures

In general, the KOOS and IKDC Subjective values decreased a bit from baseline to 3 months post surgery, then increased toward the 3-year time point, after which they very slightly declined toward the 5-year time point (Fig. and Tables 3 and 4). For both the 5 KOOS subdomains and the IKDC Subjective, the RMANOVA indicated significant differences between the score samples at the different follow-up times. All patient-reported outcome measures (PROMs) had improved over baseline with statistical significance at 1 year and onward or 3 years and onward. At 5 years the KOOS Pain improvement was no longer significant from the baseline sample.

The mean improvements over the baseline of the PROMs at 2 years, 3 years, and 5 years after surgery are given in Table 5.

There were no clear correlations between patient-reported outcomes and any of the demographic parameters, such as lesion etiology, lesion count, or lesion size. Three patients reported KOOS values of 95 or higher for all 5 subdomains from 3 years to 5 years post surgery. All 3 were male subjects, aged 45, 20, and 14 years at the time of surgery, and each had 1 lesion with a traumatic etiology (subjects 1, 9, and 11 in Table 1). Subjects 9 and 11 also reported an IKDC score of 100 from 3 years to 5 years post surgery. Of 3 other patients, the total lesion area exceeded 8 cm^2 (subjects 4, 8, and 10 in Table 1). One of them (subject 10) reported a net deterioration of KOOS Pain, Sport & Recreation, and Quality of Life over the 5-year period. This patient was male, 30 years old at the time of surgery, and had 2 lesions treated (trochlea and lateral femoral condyle). The other 2 patients (subjects 4 and 8, both male, 43 and 31 years old at the time of surgery, respectively) reported net improvements of all KOOS subdomains up to 5 years post surgery. One was treated for lesions on both the medial and the lateral femoral condyle, the other was treated for a single large lesion on the lateral femoral condyle. The patient who received the treatment bilaterally (subject 2) started reporting different KOOS and IKDC scores for the left and right knee from 3 years and onward. Her scores for the left knee were lower, with a net deterioration of the KOOS Pain and KOOS Quality of Life at 5 years post surgery.

Time of return to sports/work

All patients managed to progress to full weight-bearing within 8 weeks (2-8 weeks, according to lesion anatomic location). In general, all patients were allowed to return to office work after week 8. The time of return to work was 2 months in 9 cases and 3 months in 7 cases. All patients resumed their desired daily activities after the third month as per PROMs described in Tables 3 to 5. Sports activities were not allowed without fulfillment of the criteria described in Table 2, and not before 10 months post surgery. The time of return to sports was 10 months in 7 cases, 12 months in 3 cases, and > 12 months in 4 cases (no record for 2 cases).

Patient satisfaction

Patient satisfaction ($n = 16$ knees in 15 patients; no satisfaction record for 1 patient) at the last follow-up was: dissatisfied, 1 knee; mildly satisfied, 4 knees; much satisfied, 4 knees; very much satisfied, 7 knees. In the bilateral case, the treatment was rated dissatisfactory for the left knee and very much satisfactory for the right knee.

Discussion

Clinical performance of the treatment

In this 16-patient case series, predominantly good clinical results were obtained up to mid-term with the single-surgery treatment of symptomatic chondral lesions of the knee using intraoperatively isolated articular chondrocytes and mononuclear bone marrow cells coimplanted on a hyaluronan-based scaffold. The mean improvements of the KOOS subdomains and of the IKDC Subjective score at 5 years after surgery were all well above the minimum clinically important difference level, as determined for patients receiving

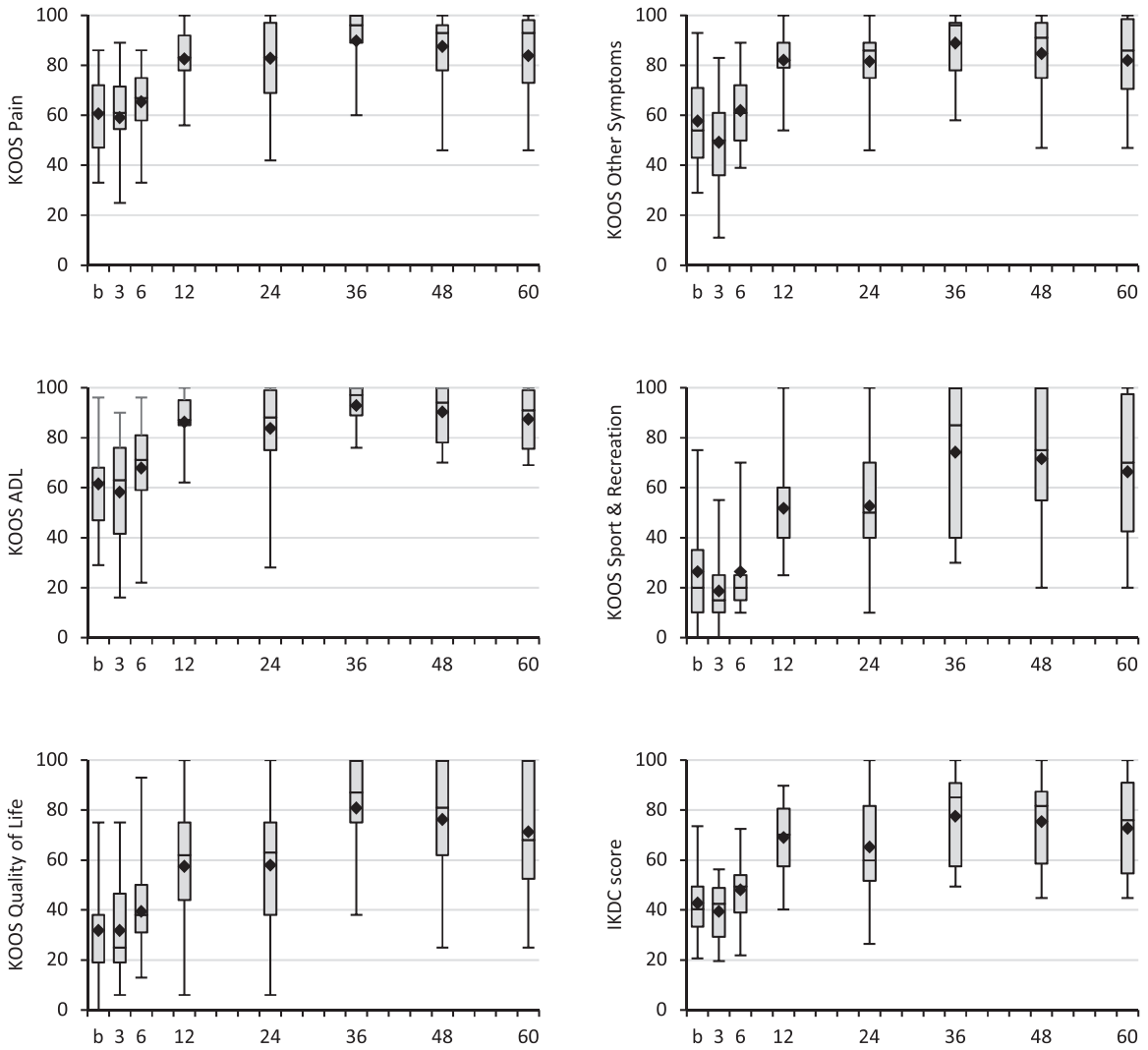

Fig. Box-and-whisker plots of the 5 subdomains of the Knee injury and Osteoarthritis Outcome Scores (KOOS) and of the International Knee Documentation Committee (IKDC) scores (third row, right side) over the 60 mo follow-up time. Diamonds represent means. The sample sizes at the different follow-up times can be found in Tables 3 and 4. ADL, Activities of Daily Living; b, baseline.

Table 3
Means ± standard deviations of the Knee injury and Osteoarthritis Outcome Scores (KOOS).

	n Knees (*n* Patients)	Pain	Other Symptoms	Activities of Daily Living	Sport & Recreation	Quality of Life
Baseline	17 (16)	60.6 ± 15.4	57.7 ± 17.5	61.6 ± 18.4	26.5 ± 23.4	31.8 ± 19.1
3 months	15 (14)	59.1 ± 18.4	49.1 ± 20.1	58.2 ± 23.8	18.7 ± 13.6	31.7 ± 18.6
6 months	17 (16)	65.4 ± 16.6	61.9 ± 14.1	67.9 ± 19.4	26.5 ± 16.7	39.4 ± 19.3
12 months	17 (16)	82.6 ± 12.2	82.1 ± 12.6*	86.3 ± 11.3†	51.8 ± 23.2	57.4 ± 26.6
24 months	17 (16)	82.8 ± 16.2	81.5 ± 14.5*	83.8 ± 18.9†	52.6 ± 26.9	57.9 ± 26.2
36 months	13 (12)	89.8 ± 14.0‡	88.9 ± 13.7*	92.9 ± 9.4*	74.2 ± 27.6*	80.7 ± 20.9*
48 months	13 (12)	87.5 ± 15.5†	84.7 ± 16.1*	90.3 ± 11.0‡	71.5 ± 29.0*	76.2 ± 23.0*
60 months	11 (10)	83.8 ± 18.6	81.9 ± 18.7*	87.4 ± 12.9‡	66.4 ± 30.7*	71.3 ± 28.7‡

Probability values for post hoc comparisons with baseline: *$P < .001$; †$P < .05$; ‡$P < .01$.

Table 4
Means ± standard deviations of the International Knee Documentation Committee (IKDC) Subjective score.

Timeline	n Knees (n patients)	
Baseline	17 Knees (16 patients)	42.7 ± 16.3
3 months	15 Knees (14 patients)	39.4 ± 12.3
6 months	17 Knees (16 patients)	48.1 ± 13.4
12 months	17 Knees (16 patients)	68.9 ± 15.4*
24 months	17 Knees (16 patients)	65.1 ± 21.5
36 months	13 Knees (12 patients)	77.5 ± 18.8*
48 months	13 Knees (12 patients)	75.2 ± 18.5*
60 months	11 Knees (10 patients)	72.6 ± 21.4*

Probability value for post hoc comparisons with baseline: *$P < .001$.

Table 5
Means ± standard deviations of the improvements over baseline of the KOOS and IKDC Subjective score at 2, 3, and 5 years after surgery.

		2 Years	3 Years	5 Years
		17 Knees (16 patients)	13 Knees (12 patients)	11 Knees (10 patients)
KOOS	Pain	22.1 ± 22.0	28.8 ± 19.0	21.5 ± 22.9
	Other Symptoms	23.8 ± 15.2	34.2 ± 14.6	31.5 ± 19.7
	Activities of Daily Living	22.2 ± 25.0	32.5 ± 18.2	26.8 ± 22.2
	Sport & Recreation	26.2 ± 30.9	49.6 ± 33.1	43.2 ± 42.1
	Quality of Life	26.1 ± 34.2	48.7 ± 28.2	40.4 ± 38.4
IKDC		22.4 ± 26.1	37.4 ± 22.9	32.8 ± 29.3

Abbreviations: IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score.

ACI.[23] In addition, the KOOS Sport & Recreation and KOOS Quality of Life improvements at 5 years were above the substantial clinical benefit (SCB) level. The IKDC Subjective improvement at 5 years (32.8) was just below the SCB level of 34.4.[23] For the KOOS subdomain Other Symptoms we could not find a minimum clinically important difference or SCB.

Two trials have explored single-stage treatments of focal knee cartilage lesions using freshly isolated autologous articular chondrocytes or chondrons: the INSTRUCT trial (NCT01041885)[7] and the IMPACT trial (NCT02037204).[6,24] The PROMs of these case series improved significantly up to 24 months, and in both series the repair tissue contained hyaline-like cartilage. The PROMs of the present case series, the IKDC, and KOOS, improved over the first 3 years after surgery and statistically remained at that improved level (except for KOOS Pain) for up to 5 years. The improvements over the baseline of the KOOS subdomains at the 2-year time point of the present study resemble those of the de Windt et al study at the 18-month time point.[6] Although in their study, the KOOS subdomains Sport & Recreation and Quality of Life featured greater improvements at 18 months, the mean lesion size in our study was greater: 4.5 ($\pm$ 2.3) cm^2 versus 3.2 ($\pm$ 0.7) cm^2. The improvements of the KOOS in both the present study and that of de Windt et al were greater than the 2-year improvements reported by Słynarski et al, who combined the primary chondrocyte approach with an osteochondral disc-shaped load-bearing scaffold that covered a mean lesion size of 2.1 cm^2.[7] Our mean improvement of the IKDC Subjective at 2 years is similar to the improvement reported by Słynarski et al: 22.4 points and 23.4, respectively.[7] At 3 years (12 patients) and 5 years (10 patients) post surgery, the improvements over baseline for all KOOS subdomains in the current study were larger than those reported for the IMPACT trial.[24]

Compared with traditional 2-stage matrix-assisted ACI (MACI), the treatment under study appears to perform mostly on par. Ebert et al treated 87 patients who suffered from isolated grade-III or grade-IV articular cartilage lesions of varying size with the MACI procedure and reported sustained improvements of, a.o., the KOOS.[25] The KOOS subdomain improvements we found resemble those reported by Ebert et al for the 2-year follow-up time point and are slightly greater at 5 years. The improvements of the KOOS subdomains of the MACI arm of the SUMMIT (Superiority of MACI Implant versus Microfractures Treatment) trial[26] were all greater than the KOOS improvements we found at 2 years. The improvements of the subdomains KOOS Other Symptoms, Quality of Life, and—to a somewhat lesser extent—Sport & Recreation we found at 5 years are on par with the MACI arm of the SUMMIT trial. KOOS Pain and Activities of Daily Life improvements at 5 years were substantially greater in the SUMMIT MACI arm.

The IKDC improvement we reported at 3 years, 37.4 points is on par with the 32.6-point improvement at 38 months reported for a 2-stage treatment in which a hyaluronan-based scaffold was cultured with autologous chondrocytes before implantation.[27] At 5 years, however, the improvement in the IKDC Subjective we found was less than the improvement achieved with the 2-surgery hyaluronan scaffold-assisted ACI: 32.8 points versus 41.1 points.[28] Although the current case series is small (16 patients, 17 knees), the above comparisons provide some indication that a single-stage autologous chondrocyte coimplantation using a hyaluronan-based scaffold may clinically perform equally to traditional 2-stage MACI in terms of patient-reported outcomes.

Single-stage chondrocyte coimplantation

ACI and MACI are 2-stage procedures that require Good Manufacturing Practices—Good Laboratory Practices cell culture facilities for the chondrocyte expansion. The field of knee cartilage repair, however, is moving toward *single-stage* regenerative techniques, to spare the patient a second surgery as well as its concomitant rehabilitation and to avoid the costs associated with laboratory cell culture. To adapt (M)ACI into a single-stage procedure, rapid high-yield isolation of articular chondrocytes is necessary. The clinical feasibility of a single-stage variant ACI was introduced by Hendriks et al in 2007.[5] The treatment explored in the current case series evolved from their rapid-chondrocyte-isolation strategy. The rapid isolation yields about 1.2 million primary articular chondrocytes and chondrons per gram of cartilage biopsy material within the hour.[19] Taking into account varying lesion sizes and an average cartilage biopsy amount of 0.7 g, this yield allows for an average chondrocyte supply of 0.3 million/cm^2 of lesion.[19] The chondrocyte supply of 2-stage ACI, however, is roughly 3 times greater and averages around 1 million culture-expanded daughter chondrocytes per square centimeter of lesion.[29] Rapidly isolated chondrocytes, however, are *primary* and have not been exposed to phenotype-shifting in vitro culture conditions.[30–32] Furthermore, combining primary chondrocytes with mononuclear bone marrow cells may augment the chondrogenicity of the chondrocytes.

In our study and in those of de Windt et al[6] and Słynarski et al,[7] primary articular chondrocytes and/or chondrons were coimplanted with a second cell type, sourced from bone marrow. Both Słynarski et al and we used freshly isolated autologous mononuclear bone marrow cells (MNCs, a heterogeneous cell population, which includes stromal and progenitor cells), whereas de Windt et al used allogeneic cryopreserved MSCs from a stem-cell bank. Through in vitro coculture research, it has been found that MSCs have a stimulatory effect on the proliferation of chondrocytes[33] and on the glycosaminoglycan production per chondrocyte,[9,34–36] while the stromal cells' own numbers decrease over time. The coculture effects involve direct cell-cell contact,[37] transfer of mitochondria,[38,39] and soluble factors such as fibroblast growth factor 1 secreted by the stromal cells.[40] Although stromal cells may exert their trophic effects on coimplanted primary chondrocytes (PCs) in vivo and in situ, no experiments have yet been reported wherein the chondrogenicity of PC coimplantation is compared with the chondrogenicity of PCs alone when seeded at the density they have in the coimplantation group. (eg, 800,000 MNCs + 200,000 PCs vs 200,000 PCs alone, within an orthotopic cartilage lesion model.) It is therefore not clear whether the PC coimplantation strategy could be equaled or outperformed by the application of PCs alone. Moreover, the minimum within-lesion density of human *primary* chondrocytes that would still support the formation of a new articular cartilage layer is unknown. In our study, the areal seeding density of primary articular chondrocytes (0.22 million/cm^2) was determined by the yield of the rapid isolation and the surface area of the lesion. Fundamental experiments using a well-defined model of a chondral lesion will be necessary to answer questions regarding the minimum volumetric or areal chondrocyte supply, the added benefit of bone marrow cells, the optimal total cell seeding density when adding bone marrow cells, and the optimal chondrocyte percentage in such coimplantation setups. Notwithstanding, individual variation between patients and between lesions, must also be assumed to have an effect on the chondrogenicity of the implanted cells.

Differences with particulated cartilage

An alternative single-stage use of autologous cartilage is the reimplantation of particulated ("minced") cartilage. The particulate is seen as a vehicle to implant chondrocytes without the need for enzymatic digestion.[41] As such, it is sometimes categorized as a fourth generation ACI variant,[42] despite important differences from ACI. One of those differences is scale. A single cartilage particle measures roughly 0.5 to 1.0 mm (there is significant variation as no standardized mincing method exists), whereas an individual chondrocyte has a mean diameter of 13 μm.[43] The dimensions of a chondron depend on the number of cells it contains, which in general is 1 to 8 chondrocytes.[43] Hence, the chondron size range is roughly 15 to 100 μm. Another important difference with ACI is that the number of viable cells contained within the particulate is unknown. In the current study and in the INSTRUCT and IMPACT trials,[6,7] the number of viable chondrocytes was counted, which allowed the cell-based intervention to be described more precisely in the patient dossier for later reference. Yet another difference with ACI is that the reimplantation of cartilage fragments introduces *two* sources of biological variation instead of one: that of the chondrocytes *and* that of the extracellular matrix.

The rationale behind implanting cartilage particulate is that chondrocytes migrate out of the particulate in situ. However, it is not known if all the chondrocytes will migrate out or a subpopulation. Neither is the long-term fate of the (partially) abandoned particulate known. Despite these unresolved questions, particulated cartilage is frequently applied, and in terms of PROM improvement, it is efficacious up to 2 years post surgery.[44–46]

Limitations

The case series included only 16 patients and a control group was absent. Although the collection of prospective magnetic resonance images is being planned, image scores are currently absent. Substantial variation existed in the size, number, and location of the cartilage lesions, as well as in the age and daily sports activities of the patients. Histological data was not collected. Also, body mass index was not monitored. We find, however, that the current results are an important addition to the still small body of knowledge on single-surgery autologous chondrocyte-based knee cartilage repair treatments.

The treatment under study has 2 main drawbacks. The first is the requirement for external cell technicians. An automated approach for cell isolations instead of a technician-based service would avoid scheduling difficulties due to technician availability. On the other hand, technicians allow for a bespoke cell isolation service (eg, the production of separate cell mixture portions proportioned to the individual lesion sizes) and continuous feedback on the process. The second drawback is the substantial tissue

processing and cell isolation time in the OR. In a number of cases it was necessary to deflate the tourniquet during the cell isolation process and later reinflate it before placement of the scaffold and cell mixture. The OR time drawback is less detrimental when the cell isolations occur in parallel with a concurrent intervention, such as an osteotomy or a ligament reconstruction (see Table 1). The drawback of an extended OR time should be weighed against the treatment being a single-stage coimplantation variant of ACI using a quantified number of nonculture-expanded autologous articular chondrocytes.

Conclusion

Coimplantation of intraoperatively isolated articular chondrocytes and mononuclear bone marrow cells on a hyaluronan scaffold is a safe and efficacious strategy in the treatment of focal symptomatic knee cartilage lesions.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Willem Cornelis de Jong, PhD, reports a relationship with Cartilage Repair Systems, LLC that includes: consulting or advisory. Leading author, Emmanouil T. Papakostas, is a member of the Editorial Board of JCJP. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Roderick B. Polak, Debbie A.E. Hendrickx, and Thomas W. Penn for their help in performing the intraoperative cell isolations.

Ethics approval

Complete written informed consent was obtained from the patient for the publication of this study and accompanying images.

References

1. Hinckel BB, Thomas D, Vellios EE, et al. Algorithm for treatment of focal cartilage defects of the knee: classic and new procedures. *Cartilage.* 2021;13(1_suppl):473S–495S.
2. Chahla J, Williams BT, Yanke AB, Farr J. The large focal isolated chondral lesion. *J Knee Surg.* 2023;36(4):368–381.
3. Hyde G, Dover S, Aszodi A, Wallis GA, Boot-Handford RP. Lineage tracing using matrilin-1 gene expression reveals that articular chondrocytes exist as the joint interzone forms. *Dev Biol.* 2007;304(2):825–833.
4. Decker RS, Um HB, Dyment NA, et al. Cell origin, volume and arrangement are drivers of articular cartilage formation, morphogenesis and response to injury in mouse limbs. *Dev Biol.* 2017;426(1):56–68.
5. Hendriks J, de Bruijn E, Schotel R, van Blitterswijk CA, Riesle J. Cellular synergy for 1 step cartilage repair [ICRS 2007, Warsaw, Poland, abstract no. 29.2]. *Osteoarthr Cartil.* 2007;15(Supplement B):B57.
6. 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 transplantation: a first-in-man trial in 35 patients. *Stem Cells.* 2017;35(8):1984–1993.
7. Słynarski K, de Jong WC, Snow M, Hendriks JAA, Wilson CE, Verdonk P. Single-stage autologous chondrocyte-based treatment for the repair of knee cartilage lesions. Two-year follow-up of a prospective single-arm multicenter study. *Am J Sports Med.* 2020;48(6):1327–1337.
8. Hildner F, Concaro S, Peterbauer A, et al. Human adipose-derived stem cells contribute to chondrogenesis in coculture with human articular chondrocytes. *Tissue Eng Part A.* 2009;15(12):3961–3969.
9. Hendriks JAA, Miclea RL, Schotel R, et al. Primary chondrocytes enhance cartilage tissue formation upon co-culture with a range of cell types. *Soft Matter.* 2010;6:5080–5088.
10. Cooke ME, Allon AA, Cheng T, et al. Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. *Osteoarthr Cartil.* 2011;19(10):1210–1218.
11. Dewan AK, Gibson MA, Elisseeff JH, Trice ME. Evolution of autologous chondrocyte repair and comparison to other cartilage repair techniques. *Biomed Res Int.* 2014:1–11. https://doi.org/10.1155/2014/27481
12. Brix MA, Stelzeneder D, Chiari C, et al. Treatment of full-thickness chondral defects with HyaloGraft C in the knee. *Am J Sports Med.* 2014;42(6):1426–1432.
13. Kon Ea, Filardo G, Condello V, et al. Second-generation autologous chondrocyte implantation: results in patients older than 40 years. *Am J Sports Med.* 2011;39(8):1668–1675.
14. Kon Eb, Filardo G, Berruto M, et al. Articular cartilage treatment in high-level male soccer players: a prospective comparative study of arthroscopic second-generation autologous chondrocyte implantation versus microfracture. *Am J Sports Med.* 2011;39(12):2549–2557.
15. Biant LC, Simons M, Gillespie T, McNicholas MJ. Cell viability in arthroscopic versus open autologous chondrocyte implantation. *Am J Sports Med.* 2017;45(1):77–81.
16. Hua Q, Knudson CB, Knudson W. Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. *J Cell Sci.* 1993;106:365–375.
17. Knudson W, Ishizuka S, Terabe K, Askew EB, Knudson CB. The pericellular hyaluronan of articular chondrocytes. *Matrix Biol.* 2019:78–79:32-46. https://doi.org/10.1016/j.matbio.2018.02.005.
18. Brittberg M, Winalski CS. Evaluation of cartilage injuries and repair. *J Bone Jt Surg Am.* 2003;85-A(Suppl 2):58–69.
19. de Jong WC, Penn TW. Single-surgery co-implantation of autologous primary articular chondrocytes with bone marrow cells in the treatment of articular cartilage lesions: observations from the operating room on tissue input and cell output. *Cartilage.* 2022;13(4):32–42.

20. Mithoefer K, Hambly K, Logerstedt D, Ricci M, Silvers H, Della Villa S. Current concepts for rehabilitation and return to sport after knee articular cartilage repair in the athlete. *J Orthop Sports Phys Ther.* 2012;42(3):254–273.
21. Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. *Health Qual Life Outcomes.* 2003;1:64.
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. Ogura T, Ackermann J, Mestriner AB, Merkely G, Gomoll AH. Minimal clinically important differences and substantial clinical benefit in patient-reported outcome measures after autologous chondrocyte implantation. *Cartilage.* 2020;11(4):412–422.
24. Saris TFF, de Windt TS, Kester EC, Vonk LA, Custers RJH, Saris DBF. Five-year outcome of 1-stage cell-based cartilage repair using recycled autologous chondrons and allogenic mesenchymal stromal cells. *Am J Sports Med.* 2021;49(4):941–947.
25. Ebert JR, Fallon M, Wood DJ, Janes GCJ. Long-term prospective clinical and magnetic resonance imaging-based evaluation of matrix-induced autologous chondrocyte implantation. *Am J Sports Med.* 2021;49(3):579–587.
26. Brittberg M, Recker D, Ilgenfritz J, Saris DBF, SUMMIT Extension Study Group. Matrix-applied characterized autologous cultured chondrocytes versus microfracture: five-year follow-up of a prospective randomized trial. *Am J Sports Med.* 2018;46(6):1343–1351.
27. Marcacci M, Berruto M, Brocchetta D, et al. Articular cartilage engineering with Hyalograft C: 3-year clinical results. *Clin Orthop Relat Res.* 2005(435):96–105.
28. Kon Ec, Di Martino A, Filardo G, et al. Second-generation autologous chondrocyte transplantation: MRI findings and clinical correlations at a minimum 5-year follow-up. *Eur J Radiol.* 2011;79(3):382–388.
29. Foldager CB, Gomoll AH, Lind M, Spector M. Cell seeding densities in autologous chondrocyte implantation techniques for cartilage repair. *Cartilage.* 2012;3(2):108–117.
30. Martin I, Vunjak-Novakovic G, Yang J, Langer R, Freed LE. Mammalian chondrocytes expanded in the presence of fibroblast growth factor 2 maintain the ability to differentiate and regenerate three-dimensional cartilaginous tissue. *Exp Cell Res.* 1999;253(2):681–688.
31. Diaz-Romero J, Gaillard JP, Grogan SP, Nesic D, Trub T, Mainil-Varlet P. Immunophenotypic analysis of human articular chondrocytes: changes in surface markers associated with cell expansion in monolayer culture. *J Cell Physiol.* 2005;202(3):731–742.
32. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. *J Orthop Res.* 2005;23(2):425–432.
33. Acharya C, Adesida A, Zajac P, et al. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol.* 2012;227(1):88–97.
34. Meretoja VV, Dahlin RL, Kasper FK, Mikos AG. Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. *Biomaterials.* 2012;33(27):6362–6369.
35. Wu L, Leijten JCH, Georgi N, Post JN, van Blitterswijk CA, Karperien M. Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. *Tissue Eng Part A.* 2011;17(9-10):1425–1436.
36. Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. *Tissue Eng Part A.* 2012;18(15-16):1542–1551.
37. de Windt TS, Saris DBF, Slaper-Cortenbach ICM, et al. Direct cell-cell contact with chondrocytes is a key mechanism in multipotent mesenchymal stromal cell-mediated chondrogenesis. *Tissue Eng Part A.* 2015;21(19-20):2536–2547.
38. Fahey M, Bennett M, Thomas M, et al. Mesenchymal stromal cells donate mitochondria to articular chondrocytes exposed to mitochondrial, environmental, and mechanical stress. *Sci Rep.* 2022;12(1):21525.
39. Korpershoek JV, Rikkers M, Wallis FSA, et al. Mitochondrial transport from mesenchymal stromal cells to chondrocytes increases DNA content and proteoglycan deposition in vitro in 3D cultures. *Cartilage.* 2022;13(4):133–147.
40. Wu L, Leijten J, van Blitterswijk CA, Karperien M. Fibroblast growth factor-1 is a mesenchymal stromal cell-secreted factor stimulating proliferation of osteoarthritic chondrocytes in co-culture. *Stem Cells Dev.* 2013;22(17):2356–2367.
41. Elias TJ, Morgan V, Chan J, Gomoll AH, Yanke AB. Cell transplantation techniques for cartilage restoration. *J Cartil Jt Preserv.* 2023;3(1):100103.
42. 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;11(1):3.
43. Hunziker EB, Quinn TM, Häuselmann H-J. Quantitative structural organization of normal adult human articular cartilage. *Osteoarthr Cartil.* 2002;10(7):564–572.
44. Cole BJ, Farr J, Winalski CS, et al. Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. *Am J Sports Med.* 2011;39(6):1170–1179.
45. Massen FK, Inauen CR, Harder LP, Runer A, Preiss S, Salzmann GM. One-step autologous minced cartilage procedure for the treatment of knee joint chondral and osteochondral lesions: a series of 27 patients with 2-year follow-up. *Orthop J Sports Med.* 2019;7(6):2325967119853773.
46. Dasari SP, Jawanda H, Mameri ES, et al. Single-stage autologous cartilage repair results in positive patient-reported outcomes for chondral lesions of the knee: a systematic review. *J ISAKOS.* 2023;8(5):372–380.