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STEM CELLS

TRANSLATIONAL AND CLINICAL RESEARCH

Allogeneic MSCs and Recycled Autologous Chondrons Mixed in a One-Stage Cartilage Cell Transplantion: A First-in-Man Trial in 35 Patients

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Key Words. One-stage • Cartilage repair • Knee • MSCs • Chondrons • Signaling • Trophic factors • Biopsy • DNA analysis

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ABSTRACT

MSCs are known as multipotent mesenchymal stem cells that have been found capable of differentiating into various lineages including cartilage. However, recent studies suggest MSCs are pericytes that stimulate tissue repair through trophic signaling. Aimed at articular cartilage repair in a one-stage cell transplantation, this study provides first clinical evidence that MSCs stimulate autologous cartilage repair in the knee without engrafting in the host tissue. A phase I (first-in-man) clinical trial studied the one-stage application of allogeneic MSCs mixed with 10% or 20% recycled defect derived autologous chondrons for the treatment of cartilage defects in 35 patients. No treatment-related serious adverse events were found and statistically significant improvement in clinical outcome shown. Magnetic resonance imaging and second-look arthroscopies showed consistent newly formed cartilage tissue. A biopsy taken from the center of the repair tissue was found to have hyaline-like features with a high concentration of proteoglycans and type II collagen. DNA short tandem repeat analysis delivered unique proof that the regenerated tissue contained patient-DNA only. These findings support the hypothesis that allogeneic MSCs stimulate a regenerative host response. This first-in-man trial supports a paradigm shift in which MSCs are applied as augmentations or "signaling cells" rather than differentiating stem cells and opens doors for other applications. STEM CELLS *2017;35:1984–1993*

SIGNIFICANCE STATEMENT

This study demonstrates the safety and efficacy of allogeneic MSCs for human use and one-stage cartilage regeneration which could lead to a cost-effective approach when compared with the current cellular cartilage repair therapy. It suggests that rather than differentiating cells that integrate in the host tissue, MSCs stimulate the patient's own cells to fill the defect and function more as stimulatory (trophic) factors.

INTRODUCTION

MSCs are known as multipotent mesenchymal stromal or stem cells. With this terminology, scientists describe the nonhematopoietic adult cell population that is present in various tissues such as bone marrow, adipose tissue, synovial membrane, and others. Their stem cell-like behavior with the capability to differentiate into different lineages of mesenchymal tissues in vitro has given rise to a new era in regenerative medicine, which aimed at regenerating tissues and organs through stem cell differentiation [1]. In the field of articular cartilage tissue engineering, successful regeneration using cultured autologous MSCs has been shown in vitro as well as in various small and large animal models [2]. A limited number of clinical trials have been reported that used autologous bone marrow- or adipose tissue-derived MSCs [3]. While clinical improvement has been shown, no studies have been able to evaluate the cell mechanisms or fate of these MSCs in vivo. The rational of using allogeneic MSCs is that the need for ex vivo autologous cell expansion would become redundant, allowing cell selection and cost effective treatment strategies. This is especially relevant, as the use of autologous cells requires a cell expansion procedure and two separate procedures. These two-stage procedures known as autologous chondrocyte implantation (ACI) have been shown to provide durable clinical improvement in randomized trials in patients with large (> 2 cm^2) articular cartilage defects [4, 5]. Indeed, since its initial description in the New England Journal of Medicine in 1994, this technique led to the first approved advanced therapy

medicinal product (ATMP) under European Union regulation [6]. Even today, variants of this technique are being introduced, most recently with use of a nasal biopsy to circumvent the need for an autologous cartilage harvest in the knee [7]. However, strict regulations, high costs, and complex logistics associated with ex vivo expansion for subsequent human implantation restrict the widespread availability of ACI [8]. While less complex surgery such as bone marrow stimulation (microfracture) is a good option for some patients, orthopedic surgeons are increasingly faced with complex patients seeking treatment for large cartilage lesions, frequently with a history of failed marrow stimulation treatment. To achieve the required tissue regeneration and clinical effect with a relatively incomprehensible cell type such as the MSC, it is of importance to understand the behavior of these cells in a clinical application. For example, while allogeneic MSCs have been found to show functional benefits from several weeks to 3 months in fracture healing [9] and myocardial infarction in animal models [10, 11], these cells seemed to disappear over time [11]. In humans, the cell fate of allogeneic MSCs remains unclear. Recently, it has been suggested that instead of differentiating into the desired tissue, MSCs are pericytes that can sense the microenvironment of the injury site and secrete site-specific factors that have reparative functions [12]. In addition to excreting these site-specific factors, MSCs have been found to have anti-inflammatory and immunomodulatory effects [13]. In fact, several clinical trials have been conducted that use of allogeneic MSCs for their immunosuppressive role [14]. The use of allogeneic MSCs is especially interesting, since their use allows for one-stage off-the-shelf application, limiting complexity, and costs.

Previously, we have shown safety and efficacy in a preclinical (compared with microfracture) [15] and early clinical (pilot) [16] study using the investigator driven Instant MSC Product accompanying Autologous Chondron Transplantation (IMPACT, NCT02037204). This study provides the comprehensive description of the completed first-in-man trial in 35 patients with 18 months follow-up.

Materials and Methods

Study Design and Objectives

This was an investigator driven academically funded phase I/II prospective monocenter study, investigating the feasibility and safety of a new ATMP for large isolated articular cartilage defects in 35 patients. The primary objective of this study was aimed at investigating clinical safety and feasibility of combining allogeneic MSCs and recycled defect derived chondrons, that is, IMPACT. The other objectives were: (a) to evaluate the fate of the implanted allogeneic MSCs, (b) to measure the level of clinical improvement, and (c) to evaluate parameters that may be indicative of structural articular joint surface repair.

Study Enrollment and in- and Exclusion Criteria

Patients were assessed for eligibility at the Department of Orthopedics, Mobility Clinic, an academic expert center for regenerative therapies and sports medicine at the University Medical Center Utrecht. The inclusion criteria were: patients (18–45 years of age) with a symptomatic isolated full-thickness cartilage defect of 2 to 8 cm^2 in the femoral condyle or trochlea, with at least 50% of functional meniscus and a stable, well aligned knee. Exclusion criteria were: signs of osteoarthritis on X-ray, concomitant diseases that may have affected the joint (e.g., rheumatoid arthritis), malalignment of the knee requiring correction osteotomy, previous surgeries in the affected knee 6 months before inclusion, (possible) pregnancy or breast feeding, and anxiety for magnetic resonance imaging (MRI) or needles.

Surgical Procedure and ATMP Manufacturing Process

Surgery was performed through a mini-arthrotomy of the knee. Cartilage defects were debrided as described for traditional cartilage repair surgery carefully removing the calcified cartilage layer and creating stable rims. The knees were temporarily closed with a sterile dressing. The resulting debrided cartilage tissue was recycled using a rapid enzymatic isolation protocol to obtain 100,000–400,0000 chondrons (chondrocytes with their pericellular matrix), as counted using 3% acetic acid with methylene blue. Allogeneic cryopreserved passage 3 bone marrow-derived MSCs, classified as ATMPs and manufactured in the GMP-licensed Cell Therapy Facility of the University Medical Center Utrecht were obtained from healthy donors as approved by the Central Committee on Research Involving Human Subjects (CCMO) (Biobanking bone marrow for MSC expansion, NL41015.041.12) [17, 18]. These MSCs were isolated from surplus bone marrow from two patients (age 2 and 5 years) originally obtained during general anesthesia aimed at hematopoietic stem cell transplantation. Consent of parents or legal guardians was given as approved by the CCMO. The bone marrow aspirates were density separated, and MSCs were isolated by plastic adherence as described previously [16] Cell viability and fulfillment of the release criteria of MSCs were assessed according to the European Pharmacopeia and in accordance with the criteria as described by Dominici et al. [19]. After thawing, MSCs were washed in 0.9% sodium chloride/10% human serum albumin with a concentration of dimethyl sulfoxide $< 0.001\%$ in the end product. Autologous chondrons and allogeneic MSCs were combined in a 10:90 ratio (standard yield) or 20:80 ratio (high yield), depending on the amount of chondrons isolated [15, 16]. Cells were suspended in fibrin glue (Beriplast, CSL Behring) using 1.5–2 million cells per milliliter. After approximately 90 minutes, the knee was reopened and the cells implanted using the fibrin glue carrier. Approximately 0.9 ml cell product per square centimeter defect was implanted. The knee was put through a manual range of motion test during surgery to ensure adherence of the IMPACT implant before the knee was closed in layers. The procedure is illustrated in this animation (https://www.youtube.com/watch?v=S3rIBjA03AA).

Rehabilitation

All patients were dismissed 1 day after surgery and followed the same standardized phased rehabilitation protocol supervised by their own physiotherapist and adjusted to individual goals [16, 20]. All patients were non weight bearing for 3 weeks with a gradual increase to full weight bearing at 9 weeks. Progression was monitored by a central study physiotherapist. High impact sports were not allowed for 9 months.

Follow-up

Adverse Events and Safety Assessment. All patients were monitored for inflammation and signs of a foreign body response by an independent physician (rheumatologist) using standardized clinical measures, pain assessment by numeric rating scale (NRS) for pain and blood analysis including serum

C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and leukocyte count. A data safety monitoring board (DSMB) was assembled in agreement with the CCMO and included an orthopedic surgeon, a professor in experimental rheumatology, and a statistician. The DSMB periodically reviewed all patient data and made recommendations concerning the continuation, modification, or termination of the trial.

Patient Reported Outcome. All patients completed patient reported outcome measures including the Knee injury and Osteoarthritis Outcome Scoring (KOOS), the visual analogue scale (VAS) for pain and the EuroQoL 5-Dimension Health Questionnaire (EQ5D) at baseline (before IMPACT therapy), and at 3, 6, 12, and 18 months follow-up. The KOOS has been validated to assess the clinical improvement after cartilage regeneration [21]. The EQ5D is a widely used health-related quality of life (QoL) measure that contains five domains, namely, mobility, self-care, usual activities, pain/discomfort, and anxiety/depression and includes a VAS for overall health [22]. It has been shown to be applicable to, and valid for, a wide range of health conditions and treatments [23–25].

Magnetic Resonance Imaging

A baseline and follow-up MRI scan (12 months) was used after surgery to assess structural repair. All MRI scans were performed on a 3-T clinical magnetic resonance (MR) scanner using a 12 channel dedicated knee coil (Achieva, Philips Healthcare, Best, the Netherlands). Morphological images were acquired in both coronal and sagittal plane using a fat suppressed (Spectral Presaturation with Inversion Recovery) Proton Density weighted sequence (PDW SPIR). For quantitative analysis, a T1rho sequence was obtained in sagittal plane [26]. The imaging parameters for PDW SPIR sequence were: repetition time (TR)/echo time (TE) = 3,860/34 ms, flip angle = 90 deg, field of view (FOV) = 160 mm, slice thickness = 2.7 mm, matrix size = 512 × 512. The imaging parameters for the 2D T1rho sequence were: TR/TE = 5.3/2.8 microseconds, flip angle = 10 deg, FOV = 140 mm, slice thickness = 4 mm, matrix size = 256 × 256, spin-lock pulse duration (TSL) = 0, 10, 20, 30, and 40. For quantitative analysis, T1rho values were calculated for the regenerated cartilage (RC) and the adjacent healthy cartilage (HC) pre- and postoperatively. To standardize T1rho values, RC and HC (RC/HC) ratios were obtained. T1rho ratios were correlated to KOOS and VAS pain scores using Pearson's correlation.

Second-Look Arthroscopy

Patients were asked consent to perform a second-look arthroscopy 1 year after surgery and were asked permission to take a biopsy from the center of the repair tissue. The International Cartilage Repair Society (ICRS) macroscopic evaluation system of cartilage repair was used to evaluate the macroscopic appearance of the repair tissue and degree of defect repair and integration with surrounding native tissue [27, 28]. A 2-mm diameter full-thickness biopsy was taken from the centre of the repair tissue. A small piece of the cartilage part of the biopsy was processed for DNA analyses, the remaining full-thickness part (cartilage and bone) was formalin-fixed and paraffin-embedded for (immuno) histological analyses.

Histological Analysis

Histological analyses were performed on 5 μM sections of full-tickness formalin-fixed paraffin-embedded biopsies [16]. Briefly, general morphology and proteoglycan deposition were assessed using a Safranin-O staining (0.125% Safranin-O (Merck, Germany counterstained with Weigert's hematoxylin [Klinipath, the Netherlands] and 0.4% fast green [Merck]). Collagen deposition was determined using types I and II collagen immunostainings (rabbit-anti human type I collagen, 1/400 dilution in phosphate-buffered saline (PBS)/bovine serum albumin (BSA)-5%, AB138492, Abcam, Cambridge, UK; mouse-anti human type II collagen, II-II6B3, 1/100 dilution in PBS-BSA-5%; Developmental Studies, Hybridoma Bank; horseradish peroxidase-conjugated anti-mouse secondary antibody (1/100 dilution in PBS-BSA-5%), visualized using 3,3′-diaminobenzidine (Sigma-Aldrich). All samples were processed and stained using the exact same procedure (e.g., color baths). Samples were scored using the ICRS II histological scoring system [29, 30]. Isotype controls for types II and I collagen immunostainings are provided in Supporting Information Figure S1.

STR Analysis

Genomic DNA was isolated from three relevant sources: the cartilage part of the 1-year central repair tissue biopsies, the recycled autologous chondrons or blood from the patients and from the donor MSCs. The loci D2S1360, D7S1517, D8S1132, D9S1118, D10S2325, D11S554, D12S391, MYCL, P450CYP19, and SE33 were amplified and sequenced [27], and specific alleles for donors and patients were determined. To identify the cellular composition, the lengths of the short tandem repeat (STR) amplicons found in the repair tissue biopsies were compared with the lengths of the amplicons measured from the MSC donors and the recipient patients. The amount of DNA present for each donor and patient in the genomic DNA isolated from the biopsy was calculated from the areas of the electropherogram from which the ratio between two cell types could be calculated.

Statistical Analysis

Predefined statistical analyses were performed with SPSS version 21.0 (IBM, Chicago, IL). A repeated-measures analysis of variance was used to test for differences in clinical outcome between baseline and 3, 6, and 12 months after surgery, an independent samples *t* test was used to test for differences in outcome between the standard and high yield. A clinical immune/rheumatologist independent of the design and surgical treatment team performed the clinical monitoring. The MRI and histological (ICRS II) grading were performed by two independent observers blinded for patient demographics and clinical outcome scores.

Results

Baseline Characteristics

The mean age of the 35 patients (24 males, 11 females) was 30 ± 8 years. Articular cartilage defects were located on the medial femoral condyle ($n = 17$), lateral femoral condyle ($n = 12$), and

trochlea ($n = 6$). The mean defect size was 3.2 ± 0.7 cm^2. Previous surgeries were performed in 20 patients. These included partial meniscectomy ($n = 6$), debridement ($n = 4$), and bone marrow stimulation by microfracture ($n = 10$). Seventeen patients received the 10:90 and 18 patients the 20:80 cellular mixture. No difference in demographic data was found between the high and low yield group, respectively. The demographics and baseline characteristics are presented in Table 1.

Table 1. Summary of the demographics and baseline characteristics ($n = 10$)

Characteristic	
Mean age in years (SD)	30 (8)
Male (*n*)	24
Mean length (m)	1·79 (0·1)
Mean weight (kg)	79·9 (12)
Previous knee surgery $n = 0$ (*n*)	15
Previous knee surgery $n = 1$ (*n*)	12
Previous knee surgery $n = 2$ (*n*)	5
Previous knee surgery $n = 3$ (*n*)	3
Defect size postdebridement (cm^2) (SD)	3·2 (0·7)
Defect location	
Medial femoral condyle	17
Lateral femoral condyle	12
Trochlea	6
Standard yield IMPACT treatment (*n*)	17
High yield IMPACT treatment (*n*)	18
Concomitant defect treated during surgery (*n*)	0
Concomitant meniscal damage (*n*)	2

Abbreviation: IMPACT, Instant MSC Product accompanying Autologous Chondron Transplantation.

Adverse Events and Safety Assessment

No signs of a foreign body response were identified by the independent rheumatologist. An increase in serum CRP levels 1 day after surgery was seen in all patients, typical for a post-surgical procedure response. One week postoperatively, the CRP levels were decreased to normal levels (Fig. 1A). The serum ESR and leukocyte count were low and remained stable over the study time points (Fig. 1B, 1C). Two patients showed an increase in serum CRP levels in the first weeks after treatment without signs of a foreign body response. After 1 week, both values were normalized (Fig. 1A). The NRS for pain decreased 1 week postoperatively and remained low compared with baseline ($p < .0001$) (Fig. 1D). No suspected unexpected serious adverse reaction were found and no re-interventions were performed. After each meeting with the DSMB, study continuation was advised. Adverse events included post-surgery and rehabilitation symptoms without concerns for a disproportional host response (Table 2).

Clinical Outcome

The mean improvement in the Knee injury and Osteoarthritis Outcome Score (KOOS) showed a gradual positive change from

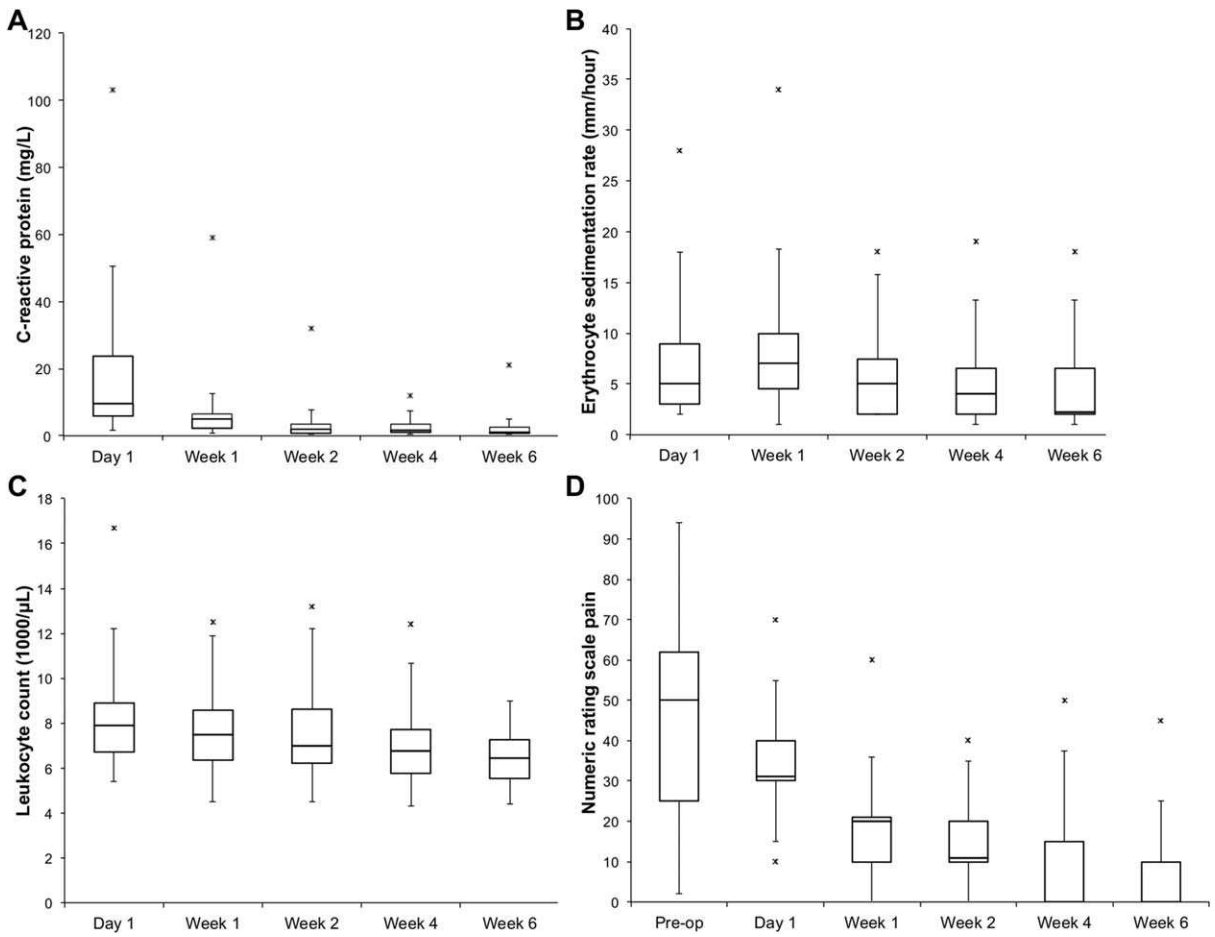

Figure 1. Safety assessment. Box-plots showing serum levels of C-reactive protein **(A)**, erythrocyte sedimentation rate **(B)**, leukocyte count **(C)**, and numeric rating scale for pain **(D)** at day 1 and 1, 2, 4, and 6 weeks after surgery. Outliers are shown by x. ($n = 35$).

baseline to 18 months with the largest effect at 3 months follow-up for all subscores. The mean overall KOOS showed an improvement from 57.9 ± 16.1 to 76.6 ± 11.1 ($p < .0001$) at 3 months and 85.4 ± 13.3 ($p < .0001$) at 18 months follow-up. Statistically significant improvement ($p < .000002$) in all subscales was seen with the biggest effect in the Sports and Recreation subscale (mean baseline score: 32.3 ± 22.3, mean 18 month score: 73.2 ± 24.1) ($p = .00000001$). No significant difference in KOOS clinical outcome scores ($p = .94$) and VAS pain scores ($p = .58$) were found between the standard yield and high yield groups. All patients showed a statistically significant reduction in mean VAS pain score from baseline (45.3 ± 24.2) to 18 months after surgery (9.7 ± 15.4 [$p < .00000001$]). The clinical outcome scores are presented in Figure 2.

Magnetic Resonance Imaging

MRI scans indicated complete filling of the defect, integration with the host tissue, and attachment to the subchondral bone at 12 months compared with baseline. Worst, mean, and best proton density (PD) images ($n = 35$) are provided in Supporting Information Figure S2. The mean T1rho values were 43.1 ± 6.9 for HC and 47.9 ± 13.5 for RC 12 months after surgery. There was no significance difference between T1rho values of the RC and HC at 12 months (mean difference T1rho; 4·7 ± 11·3, CI: 0·57–8.9, $p > .05$). An example of a T1rho map and the T1rho values are presented in Figure 3A, 3B, respectively. Correlation analysis showed a moderate correlation ($R = -0.46$, $p < .05$) between T1rho RC/HC ratio and VAS pain 12 months after surgery and no correlation with the KOOS ($p > .05$).

Table 2. Treatment-related adverse events

Adverse event	*n*
Patients with at least one adverse event	46
Post-surgery (24 hours)	
Nausea and vomiting	6
Urinary retention	2
Other (e.g., headache, vasovagal episode, etc)	10
Musculoskeletal	
Arthralgia	13
Joint swelling	8
Crepitations	5
Increased serum CRP levels	2
Giving way sensation	1
Second lesion (incidental finding second-look arthroscopy)	1
Total	48

Abbreviation: CRP, C-reactive protein.

Second-Look Arthroscopy

A second-look arthroscopy at 12 months follow-up was performed in 33 patients, two patients did not give their consent. The defects were filled with repair tissue which showed good integration with the native tissue. Each graft was manipulated with an arthroscopic probe and showed no signs of loosening. (Supporting Information Video). The repair tissue of was grade I (normal tissue) in the majority of patients ($n = 22$) and grade II (nearly normal tissue) in 11 patients as scored by macroscopic ICRS score.

Histology

A total of 32 biopsies could be used for histological analysis. The repair tissue was rich in proteoglycans as shown by Safranin-O staining on the biopsies (Fig. 4). Both types I and II collagen were deposited in the repair tissue, but the intensity for type II collagen was stronger compared with type I collagen (Fig. 4). The ICRS II histological scores for the observers were good with a mean overall score of 70 (±14·3) (Table 3). The ICRS II scores, along with the defect size and cell ratios are provided in Table 3. Four cases were selected based on their ICRS II scores (worst to best, Table 3). The corresponding macroscopic images and histological stainings are presented in Figure 4. Supporting Information Table presents all ICRS II scores along with the description of the subscales. Two biopsies showed patches of proteoglycans as indicated by Safranin-O and mainly type I collagen immunostaining instead of type II collagen. All other biopsies showed a stronger type II collagen immunostaining. No correlation was found

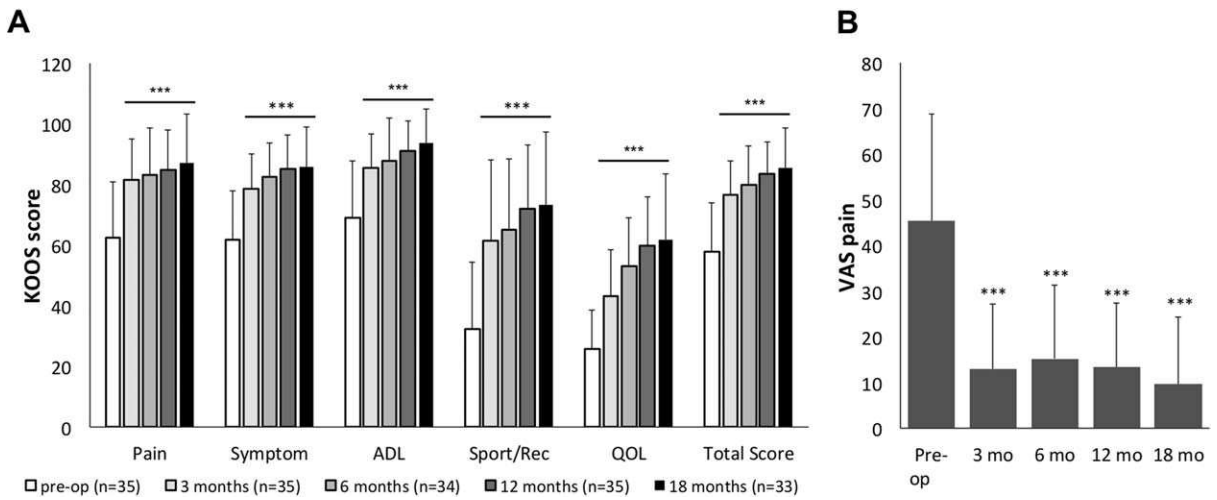

Figure 2. Clinical outcome scores. Patient reported outcome scores: the Knee injury and Osteoarthritis Outcome Score (KOOS) **(A)**, subscales for pain, symptom, activities of daily living, sport, and recreation (sport/rec), quality of life and overall score, and visual analogue scale for pain **(B)** from preoperative (preop, $n = 35$) to 3 ($n = 35$), 6 ($n = 34$), 12 ($n = 35$), and 18 ($n = 33$) months post-surgery. Data is presented as mean ± SD. ***, $p < .001$ compared with preoperative. Abbreviations: ADL, activities of daily living; KOOS, Knee injury and Osteoarthritis Outcome Scoring; QOL, quality of life; VAS, visual analogue scale.

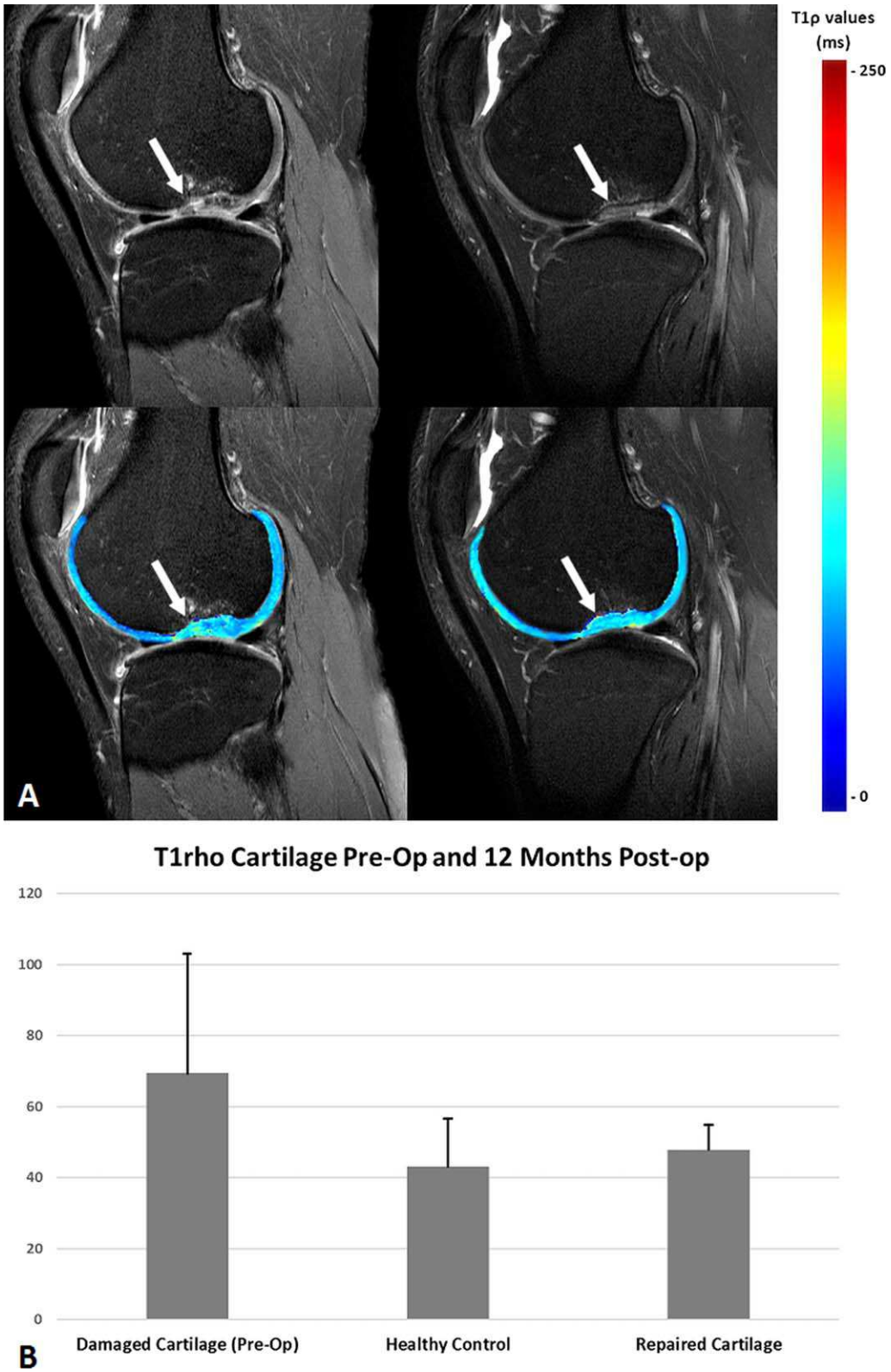

Figure 3. Biochemical MRI (T1rho) imaging. **(A):** T1rho values for femoral cartilage segmentation are superimposed on pre- and post-operative anatomical magnetic resonance imaging (Proton Density weighted sequence Spectral Presaturation with Inversion Recovery) images ($n = 35$). The color bar shows the range of T1rho values with low values indicating healthy cartilage (HC). **(B):** Mean T1rho values at the cartilage defect site pre- and postoperatively compared with adjacent HC. There was no significant difference in Th1rho value between the repaired tissue and adjacent HC 12 months after surgery.

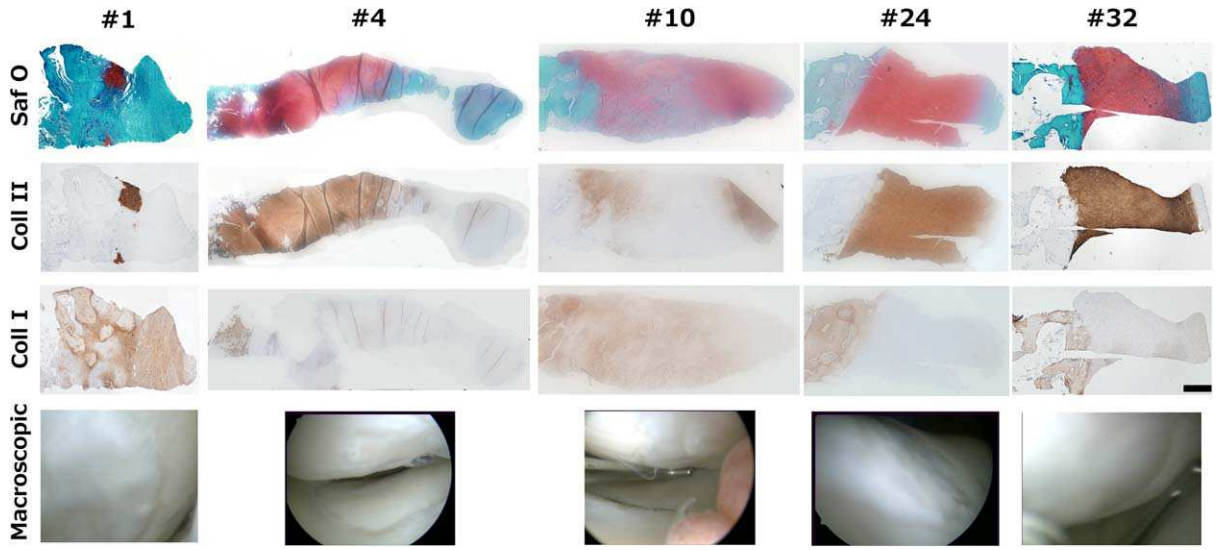

Figure 4. Histolofical analysis. Safranin-O proteoglycan staining (best, mean, worst), and types II and I collagen immunostaining (Coll II and I) on biopsies ($n = 32$) from the centre of the repair tissue 12 months after surgery, with the subchondral bone on the left side and cartilage surface on the right. Four cases were selected based on the worst to best (left to right) International Cartilage Repair Society II histological outcome scores as presented in Table 3. Scale bar = 400 μm. Abbreviations: Coll, collagen; Saf O, Safranin-O.

between the ICRS II histological scores and defect sizes or the percentages of chondrons.

STR Analysis

At least seven loci could be used to define the origin of the genomic DNA from the biopsies. For both the 17 patients treated with the standard yield (10:90 ratio) and 18 patients treated with the high yield ratio (20:80), the biopsies contained only autologous DNA. Thus, no DNA of the allogeneic MSCs could be detected at the detection limit of the assay (1 in 100,000 cells).

Discussion

This is the first study showing safety and efficacy of the proof of concept that allogeneic MSCs augment one-stage articular cartilage repair. It demonstrates that a cartilage cell therapy using rapidly isolated autologous chondrons recycled from the rim of the cartilage defect combined with allogeneic human bone marrow-derived MSCs is feasible, safe, and allows for improvement of clinical outcome and tissue repair. Using DNA analysis, this study provides evidence that MSCs do not engraft in the host tissue as previously suggested by others [28]. The biopsy was taken from the center of the repair tissue in each defect. Cells were homogeneously mixed and implanted as uniform suspensions. However, it cannot be excluded that a sampling error could play a role in our results. It could also be that a non- detectable immune response removed the MSCs from the joint. In a small animal myocardial infarction model, allogeneic MSCs have been suggested to differentiate and evoke an immune response, while still giving functional benefits [11]. Microarray assays and enzyme linked immunosorbent assays identified multiple protective factors that were expressed and excreted by the MSCs [11]. The question rises, however, whether reimplantation of allogeneic MSCs for tissue repair strategies will activate a memory T-cell response. Still, it seems most likely that MSCs influence joint homeostasis by evoking a temporary stimulatory response before disappearing from site [31] Again, it seems safe to presume that if the allogeneic MSCs still played a role in the final tissue formation, we would have identified donor DNA as assessed by the many STR repeats at the detection level of 1/100,000 cells. Eighteen months after surgery, no symptoms were identified that would indicate MSC engraftment at different sites such as the bone marrow, lungs, or liver. Although this has been described previously for small animal models, microchimerism and unwanted migratory behavior from the sparsely vascularized joint seems unlikely, but cannot be ruled out completely [32] Nevertheless, our study and other trials exploring the use of allogeneic MSCs found no sign of such events [2]. Our in vitro studies on cocultures of chondrocytes and MSCs have shown that even without immune cells present, MSCs disappear from the cultures while chondrocytes proliferate [33]. Cell–cell contact was one of the primary indicators for tissue regeneration. Others have also shown a trophic or signaling role of MSCs, both in an immunomodulatory and regenerative role [34]. This is in contrast to the more traditional view on MSCs as stem cells with multipotent differentiation capacity [12]. While here it seems likely that MSCs have a signaling or trophic role in vivo, a cell tracking and real time trophic factor analysis would be necessary to confirm this hypothesis.

The results of this study indicate that using a mixture of autologous and allogeneic cells is feasible and could be a safe efficient and more cost effective strategy. Such a one-stage procedure, with “off-the-shelf” use of allogeneic MSCs would have major benefits for patients, payers and providers alike as they would not need two separate surgical treatments. In addition, patients can start rehabilitation immediately following surgery, instead of having to wait on a cell expansion period. Other strategies for a combined one-stage cell therapy would lie in a combination of autologous cartilage cells combined with enriched stem cell products such as bone marrow concentrate, the mononuclear fraction of bone marrow or

Table 3. Defect size, cell ratios, and the corresponding ICRS II histological outcome score

Patient ID	Defect size (cm^2)	Ratio chondrons: MSC implanted	Ratio chondron: MSC 12 months	ICRS II microscopic score[a]
1[b]	4.5	10: 90	100: 0	46
2	3.0	10: 90	100: 0	50
3	3.0	10: 90	100: 0	51
4[b]	4.0	20: 80	100: 0	60
5	2.5	20: 80	100: 0	61
6	3.0	20: 80	100: 0	62
7	3.0	20: 80	100: 0	64
8	2.7	10: 90	100: 0	65
9	3.0	10: 90	100: 0	66
10[b]	3.0	20: 80	100: 0	71
11	2.7	10: 90	100: 0	72
12	4.5	10: 90	100: 0	72
13	4.0	20: 80	100: 0	73
14	2.7	20: 80	100: 0	74
15	2.7	20: 80	100: 0	74
16	2.7	20: 80	100: 0	75
17	5.0	10: 90	100: 0	77
18	3.4	10: 90	100: 0	78
19	3.0	20: 80	100: 0	78
20	2.0	20: 80	100: 0	78
21	3.5	10: 90	100: 0	79
22	3.5	10: 90	100: 0	79
23	2.7	10: 90	100: 0	79
24[b]	3.5	20: 80	100: 0	80
25	4.5	20: 80	100: 0	81
26	2.7	10: 90	100: 0	82
27	3.0	20: 80	100: 0	82
28	3.0	20: 80	100: 0	82
29	2.5	10: 90	100: 0	82
30	2.5	10: 90	100: 0	84
31	2.7	10: 90	100: 0	85
32[b]	2.7	10: 90	100: 0	89

Abbreviations: ICRS II, International Cartilage Repair Society II, MSC, mesenchymal stem cell.

[a]Corresponding macroscopic and histological pictures are provided in Figure 4.

[b]Representing the ICRS II scores as completed by two independent observers blinded for patient demographics, magnetic resonance imaging images, and clinical outcome scores. ICRS II scores and a description of the subscales are presented in the Supporting Information Table.

vascular stromal fraction of adipose tissue. However, if the sole function of the MSCs is to provide stimulatory factors, it can be suggested that a higher amount of MSCs might be more beneficial compared with enriched products. More studies are needed to confirm this. The advantage in terms of tissue regeneration of using freshly isolated chondrons instead of expanded chondrocytes has been shown previously [35] Here, the improvement in tissue regeneration and interaction with MSCs is attributable to the intact pericellular matrix, which is lost to the utmost extent during cellular expansion [36, 37]. An additional advantage of using recycled chondrons is that donor-site morbidity is prevented [38] Furthermore, their regenerative capacity compared with non-weight bearing HC has been shown [39] A first-in-man trial just recently published in the Lancet used the nasal septum as a cell-source for cartilage repair treatment [7]. While this study neatly circumvents donor-site morbidity to the knee, the limits of a complex cell expansion procedure with the patient needing to undergo two separate procedures separated by several weeks are a cause for concern. Again, a cost-effective single-stage cell-based procedure allowing treatment of large and complex cartilage lesions would be an innovation of value for the growing patient population and field of regenerative medicine [8].

As we have demonstrated safety and feasibility of the current approach, we can now make predictions on clinical efficacy that allow for power calculations for a Phase III/IV trial. In the future, a randomized approach compared with a conservative treatment group or a relevant comparator will be of interest. This is underlined by the consistent statistically significant improvement found in clinical outcome as well as the correlation shown between pain and the biochemical (MRI) quality of the repair tissue. However, further research into the value of T1rho assessment of cartilage repair tissue is warranted as the sample size is small, and correlation between MRI and clinical outcome after cartilage repair has been proven difficult to, although it seems biochemical imaging is more promising in its predictive value compared with morphological imaging [40, 41].

Our previous in vitro and in vivo studies, have shown an advantage of using a combination of chondrons and MSCs when compared with chondrons or MSCs alone, making it unlikely the same results would be achieved with the limited available autologous chondrons isolated [15, 33]. Others have consistently corroborated the advantage of combining chondrocytes and MSCs compared with chondrocytes alone [42].

In this study, structural evaluation after 12 months using both biochemical MRI scans and second-look arthroscopies showed hyaline cartilage-like tissue repair with good integration with the native tissue. The quality of the repair tissue was found to be similar or even superior to the histological results shown after ACI, with only two biopsies showing mainly fibrocartilage (mainly type I collagen), while these patients were found to strongly improve in clinical outcome scores [29, 43]. Our results confirm a positive effect on short-term cost-effectiveness compared with ACI as we have previously modeled in an early health technology assessment [44]. Especially, since we found non-inferior and even superior clinical outcome compared with ACI and mirofracture in comparison with previous randomized controlled trials [45, 46]. Ongoing work aims at designing a (closed) system with shorter treatment times allowing broader availability and improved efficiency. The underlying cellular mechanisms as well as the comparison with current or developing technologies should be explored in future clinical trials that investigate the regenerative or augmentative capacity of MSCs.

Conclusion

The findings of this unique first-in-man study demonstrate that allogeneic MSCs can be a safe cell source to augment or facilitate tissue regeneration in a clinical setting. Instead of engraftment or differentiation as previously suggested, allogeneic MSCs seem to stimulate tissue regeneration through paracrine mechanisms and cellular communication.

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Author Contributions

T.S.d.W.: inventor, conception and design, patient care, provision of study material/patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; L.A.V.: inventor, conception and design, provision of study material, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; I.C.M.S.-C.: conception and design, provision of study material, collection and assembly of data, manuscript writing, final approval of manuscript; R.N.: patient care, provision of study material/patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; M.H.P.v.R.: provision of study material/patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; D.B.F.S.: inventor, conception and design, surgeries, patient care, provision of study material/patients, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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