Early Efficacy of Type I Collagen Based Matrix-Assisted Autologous Chondrocyte Transplantation to Treat Articular Cartilage Lesions

Xiang Li

Shanghai Changzheng Hospital

Jiatian Qian

Shanghai Changzheng Hospital

Shiao Li

Shanghai Changzheng Hospital

Peiliang Fu (✉ fupeiliang@163.com)

Shanghai Changzheng Hospital <https://orcid.org/0000-0002-5950-5308>

Chengyan Chen

Shanghai Changzheng Hospital

Research article

Keywords: cartilage lesion, autologous chondrocytes, early efficacy

Posted Date: May 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-528457/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Purpose: To investigate the clinical, radiological, and histological results of type I collagen-based matrix-assisted autologous chondrocyte transplantation (MACT) in the treatment of chondral lesions of the knee.

Methods: The study prospectively enrolled 20 patients with symptomatic knee chondral defects (mean size defect was $2.41 \pm 0.43 \text{ cm}^2$, range 2.0 to 3.4 cm^2) in the lateral femoral condyle and femoral groove who underwent type I collagen-based MACT between July 2017 and July 2019. KOOS was assessed preoperatively, with periodic clinical follow-up performed preoperatively and then every 3 months for up to 12 months postoperative period, and thereafter at 1-year intervals. During this follow-up, serial magnetic resonance imaging T2 mapping of repair cartilage was used to reflect the quantitative analysis quality of the regenerative cartilage. In one patient, second-look arthroscopy was performed at 12 months after implantation to assess the characteristics of cartilage regeneration.

Results: Compared with preoperation, the score of the pain, symptoms, activities of daily living, sports and recreation, and quality of life showed statistically significant improvement with a significant difference at 3, 6, 12, and 24 months after operation ($P < 0.05$). The difference in KOOS subscales scores between every two-time point was statistically significant ($P < 0.001$). HE stains showed the newly formed cartilage was naive chondrocytes. Safranin O-fast green stain manifested in the regenerated tissue comprising predominantly fibroblast-like cells surrounded by glycosaminoglycans. Immunohistochemistry analysis showed that the expression of collagen type II was more clearly and evenly distributed than collagen type I.

Conclusion: Type I collagen-based MACT was a clinically effective treatment for functional and pain level improvement, and this method presented histologic evidence of inducing hyaline-like cartilage in cartilage lesions by biopsy in one case. The quantitative MRI T2-mapping test showed that there was a difference between the transplanted cartilage and the surrounding hyaline cartilage.

Introduction

Hyaline cartilage has a unique capacity to cope with pressure transformations, and it is thus vital to the proper function of the musculoskeletal system. Unfortunately, its lesions rarely heal spontaneously because of avascular and aneural surroundings limiting the healing capacity[1]. Patients with symptomatic lesions will often endure long-standing activity-related knee pain and swelling. It has introduced several methods to treat high-grade cartilage defects in young active patients comprising microfracture, osteochondral autograft transfer, or mosaicplasty, and osteochondral allograft [2]. None of the above methods can completely regenerate cartilage with good biological functions[3–5].

The most promising method to make a breakthrough in the limited intrinsic healing potential of articular hyaline cartilage is autologous chondrocyte implantation (ACI)[6]. If chondrocytes are applied onto the damaged area in combination with a membrane, such as the tibial periosteum or biomembrane, or

preseeded in a scaffold matrix, this technique is called ACI. First-generation ACI contains the cultured chondrocyte solution within the lesion site with a periosteum flap. Due to using the autologous periosteum flap derived from a separate tibial incision, this method can lead to a longer operation time and potential graft hypertrophy[2, 7, 8]. Second-generation ACI method, which relies on the use of collagen membranes, has demonstrated more advanced regenerative potential[8]. Furthermore, concerns mount over the uneven distribution of chondrocytes within the lesion and the potential risks of cell leakage, and these problems are still unresolved. Since the monolayer expansion chondrocyte leads to the hindered reconstruction of the cartilage-specific matrix, autologous cell-based cartilage therapies are challenged by chondrocyte dedifferentiation [9]. As we already know, that cell-to-cell contact promotes chondrogenic differentiation, and three-dimensional culturing can be used to induce redifferentiation of monolayer-expanded autologous chondrocytes[10]. Third-generation ACI is known as a matrix-associated ACI (MACI), and the three-dimensional cell culture is more capable of mimicking the microenvironment of cell growth in vivo[11].

Based on the preclinical studies, we reported the initial description of the effects of using three-dimensional type I collagen combined with autologous chondrocytes to treat cartilage lesions as the primary outcome, and we investigated the efficacy of such procedures by the morphologic quality of the repair tissue with magnetic resonance imaging (MRI) and histologic analyses of the repair tissue as secondary outcomes.

Methods

Study Design and Patients

An observational study limited to 20 cases (8 females and 12 males) was approved by the Science and Technology Committee of Shanghai and performed at the hospital between July 2017 and July 2019. Twenty patients aged 20 to 50 years (mean age 40.05 ± 8.03) were enrolled with isolated full-thickness cartilage defects of the knee (measuring more than 2 cm in diameter, mean size 2.41 ± 0.43 , ICRS grade III or IV, 10 Groove and 10 lateral femoral condyles). The inclusion and exclusion criteria for patient selection were detailed in Table 1.

Table 1
Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
☒Traumatic cartilage injury (ICRS grade III or more, measuring more than 2 cm in diameter)	☒The presence of an active infection or inflammatory disease such as rheumatoid arthritis
☒High-grade femoral condylar cartilage lesions with or without shallow subchondral bone involvement	☒Patient unable or unwilling to comply with the long rehabilitation process/activity restrictions recovery
☒Recalcitrant with more than 6 months of conservative care	☒Unaddressed causes of increased loading (knee coronal alignment, meniscal deficiency, ligamentous laxity, patellar maltracking)
☒A 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 grade I and II)	☒Obesity (defined as BMI > 35 kg/m ²)
☒Desire to return to sports or high-impact activities	☒More than 50% joint space narrowing on weight-bearing radiographs
☒Younger age, 20–50 y	

ICRS-International Cartilage Regeneration & Joint Preservation Society; BMI-Body Mass Index

The current study was designed per the Declaration of Helsinki, and it was approved by the institutional review board of the Hospital (Approval number: 2018-024-021). Informed consent was obtained from all patients before enrolling in this study.

Procedures

The current type I collagen-based matrix-assisted autologous chondrocyte transplantation (MACT), known as MACI, consisted of isolating the chondrocytes in the first operation, the expansion for two weeks in vitro, cell preseed in a matrix, and the transplantation in a second surgical procedure[12].

About 150 to 200 mL of whole blood was taken from the patient for the cultivation of the implant before general anesthesia. We performed an initial arthroscopic procedure of the knee to determine the location and size of the lesion, as well as the consistency or firmness of the adjacent articular cartilage and the integrity of the menisci or ligaments. The cartilage biopsy specimen providing seed cells was harvested from a non-weight-bearing area of the knee using a Jamshidi needle biopsy guided by an arthroscope. The specimen was immediately placed in a buffered serum-free medium and then was delivered to the laboratory with hypothermic preservation at 2 to 10 degrees celsius. In the laboratory, chondrocytes were isolated from the cartilage biopsy specimen by collagenase digestion for 16 to 24 hours and obtained by density gradient centrifugation, and the obtained cell number and viability were assessed subsequently. Furthermore, the sample was preserved at 2–10°C to test quality for 2 months. Chondrocytes were suspended in a double-buffering HEPES solution, and this was gently mixed with type I collagen (6 mg/mL) from rat tails in equal parts to obtain a final concentration of type I collagen (3 mg/mL). The

collagen-chondrocyte mixture was allowed to polymerize at 37 degrees celsius in a humidified atmosphere. Each implant was made into two samples with a diameter of 30 to 40 mm and two times the height (4 to 6 mm) of the articular cartilage (2 to 4 mm) in the lesion area. The chondrocyte-seeded implants were cultured in autologous serum for a period of 10 to 14 days (37 degrees celsius, 5% CO₂), and the medium changes were carried out every 3–4 days by replacing 80 mL of medium per well. Then, it was converted to the hospital within 24 hours. Before shipment, quality control of each implant had to meet defined requirements such as cell viability higher than 80% with CellCountigKit-8(WST-8 / CCK8) or flow cytometry, cell count more than 3×10^4 measured by a grid-counting method, aseptic negative qualified by detecting mycoplasma with Real-Time Reverse Transcriptase PCR (RT-PCR) utilizing PCR Mycoplasma Test Kit (Applichem), and DNA electrophoresis employed to detect the expression of type II collagen.

Approximately 2 weeks later, a second operation was required, in which the cultured chondrocytes were reimplanted into the cartilage lesion, and they proliferated to produce a durable load-bearing regenerative tissue over time[13, 14]. The operative procedure was performed following intravenous antibiotics and exsanguination of the lower extremity using a tourniquet. On the basis of the location of the defect, a medial or lateral parapatellar mini-open arthrotomy was applied to access the lesion area. Chondral lesions were debrided down to the subchondral bone, and the edges of the lesion were trimmed using a sharp metal punch. The implants were prepared through a metal punch 2 mm wider than the corresponding punch utilized for trimming the cartilage defect. The basal part of the cartilage lesion was coated with a Porcine Fibrin Sealant Kit (Bioseal Biotech) with a specific metal spatula, as well as around the implant before it was transferred into the lesion. Because of the ability of the hydrogel to release more than 50% of its water content, the implant was fabricated twice the height of the defect and could easily be adapted to the individual shape of the cartilage lesion. In cases of osteochondral lesions, the subchondral bone was augmented with autologous cancellous bone harvested from the tibial head. Alternatively, autologous bone cylinders from the iliac crest were used to reconstruct the subchondral plate.

Outcome Measures

All patients were assessed, on a postoperative day 1, then 1, 3, 6, 12, and 24 months postoperatively, by the general condition and the knee surgical site such as pain, swelling, skin features, and infection. Furthermore, leukocyte counts, erythrocyte sedimentation rate, and serum levels of c-reactive protein were assessed on postoperative days 1 and 3, and then 1, 3, 6, 12, and 24 months postoperatively to monitor inflammatory responses.

Knee injury and Osteoarthritis Outcome Score (KOOS) was assessed preoperatively and then at 3, 6, 12, and 24 months postoperatively. The morphologic and compositional characteristics of the repair site for all patients were assessed. MRI was taken on all patients on a 3.0-T MR scanner to investigate conventional images and T2 mapping of the repaired tissues preoperatively and then at 3, 6, 12, and 24 months postoperatively. T2 mapping was obtained for all patients, and the T2 values for the repaired

tissue were measured at each time point. A second-look arthroscopy for one patient was performed at 12 months, and a 4-mm-diameter needle biopsy was taken from the center of the repair site with patient informed consent. The indication for a second-look arthroscopy was given by arthrofibrosis or meniscus lesions. This procedure was performed after the MRI examination to avoid potential influence on the MRI assessment. The morphology of the chondrocytes, the distribution of cells, and ECM synthesis were further studied by Hematoxylin and eosin (HE) stain and Safranin O-fast green (S-O) [15], and Sections of biopsy specimens were stained with immunohistochemical (IHC) to verify the existence of the Collagen Type I and Collagen Type II.

Rehabilitation Program

For the isolated femoral lesion, the knee joint was immobilized for 72 hours in a brace locked at 10 degrees of flexion after the operation. Knee flexion was limited to 30 degrees during the first 3 weeks and to 60 degrees for another 3 weeks, with partial weight-bearing for 12 weeks consisting of 15 kg for 6 weeks and 30 kg for the following 6 weeks. Nonweightbearing was encouraged for at least 6 weeks during the reconstruction of the subchondral bone that was performed. We also recommended assisted physical therapy and bicycle training after 6 weeks, and training for enhanced muscle formation started after 12 weeks. In all cases, continuous passive motion (CPM) was recommended for 6 weeks. Physical activity, such as noncontact sports activities, swimming, and biking, was allowed after 6 months. More competitive activity loads, containing soccer, track, and field athletics, were allowed 12 months after the operation.

STATISTICAL ANALYSIS

We assessed changes in clinical scores and radiologically measured cartilage properties at different follow-up times by one-way ANOVA, and the results obtained preoperatively and at each follow-up were compared. Data were analyzed with SPSS23.0 and Prism 8 software with a significance set at $P < 0.05$.

Results

Adverse Events

No serious clinical adverse events were observed up to 24 months after MACT (Table 2). Joint pain, effusion, and swelling were observed in the early stages after the operation, and 8 weeks completely improved all symptoms. No postoperative infections were observed up to 24 months after the operation for any patient.

Table 2
Adverse Events

	Patients: n	Time Point: wk
Joint pain	4	< 4
Joint effusion	0	—
Joint swelling	2	< 8
Joint stiffness	0	—
Infection at the surgical site	0	—

There are no serious adverse events among the 20 patients (ie, new cartilage lesion in the affected knee, or failure at implanted sites).

KOOS Score

Although somewhat variable among cases, it significantly improved all subcategories of KOOS by 24 months (Fig. 1). For KOOS subscales, compared with the preoperation, the KOOS pain (28.47 ± 21.09 vs 57.08 ± 15.73 , 69.17 ± 14.97 , 80.42 ± 7.67 , 89.86 ± 4.96 , $F = 56.76$, $p < 0.05$), symptoms (33.39 ± 18.26 vs 48.93 ± 15.42 , 63.57 ± 10.59 , 71.61 ± 7.09 , 84.29 ± 5.95 , $F = 50.95$, $p < 0.001$), activities of daily living (76.32 ± 6.16 vs 81.54 ± 3.98 , 86.10 ± 3.00 , 89.05 ± 2.41 , 94.63 ± 2.09 , $F = 67.24$, $p < 0.05$), Sports&Rec (34.00 ± 12.31 vs 42.00 ± 14.46 , 54.00 ± 14.38 , 66.50 ± 10.89 , 83.50 ± 5.87 , $F = 53.80$, $p < 0.05$), and quality of life (23.13 ± 12.52 vs 39.69 ± 13.64 , 51.24 ± 13.09 , 62.19 ± 12.08 , 73.44 ± 10.31 , $F = 49.84$, $p < 0.05$) showed significant improvement at 3, 6, 12 and 24 months after operation, and the difference in scores between every two-time point was statistically significant ($P < 0.001$).

MRI

In accordance with MRI assessment, cartilage lesions were filled with newly generated tissue over time, and the lesion filling rate reached 100% coverage without detectable hypertrophy of the repaired tissue by 24 months for all patients. Some subchondral bone edema was observed around the implantation sites at 3 and 6 months postoperatively, but such abnormal signals disappeared by 24 months in all cases.

The MRI demonstrated that the vast majority of the cartilage defect was filled and regenerated at 3 months after the operation, but the integration with the surrounding cartilage was not complete, and it significantly reduced the signal intensity of the repaired tissue. Compared with the results in the third month, the MRI revealed that integrating the implanted cartilage and the surrounding cartilage had made significant progress at 6 months after the operation, most of them reaching the level of complete integration, and it significantly reduced the signal of the repaired tissue, but the subchondral bone had not yet satisfactorily. At 12 and 24 months after the operation, all indicators, as well as the subchondral bone, had returned to near-normal levels. During the recovery period, the lesion site was completely repaired and integrated with the surrounding cartilage, and the signal intensity of the repaired tissue was

similar to that of the surrounding tissue and the subchondral bone (Fig. 2). The MACT applied to regenerate cartilage could quickly fill and repair the injured cartilage. It was completely integrated with the surrounding cartilage in half a year, and the repaired tissue reached the same signal intensity as the surrounding cartilage at 12 months after the operation.

At different time points, the MOCART score increased statistically significantly from 50.50 ± 6.67 at 3 months, to 77.00 ± 13.42 at 6 months, to 88.00 ± 6.37 at 12 months, and to 92.80 ± 4.98 at 24 months, and then between every two-time point was statistically significant ($F = 98.723$, $P < 0.001$) except for the 12months and 24months (Fig. 3).

MRI T2mapping

T2 mapping revealed that implanted chondral lesions were becoming similar, in intensity, to the surrounding cartilage over time (Fig. 4). At different time points, the T2 values decreased statistically significantly from 95.05 ± 5.61 preoperatively to 85.40 ± 5.33 at 3 months, to 78.05 ± 3.80 at 6 months, to 41.85 ± 4.16 at 12 months, and to 34.85 ± 2.01 at 24 months. The T2 values for the repaired tissue gradually decreased to approach near normal values eventually ($P < 0.05$), and the between every two-time point was statistically significant ($F = 976.11$, $P < 0.001$). Moreover, there was still a difference between the implanted cartilage and the surrounding normal hyaline cartilage (T2 values approximately 29.80 ± 2.12 ms) (Fig. 4).

Second-look

The repaired tissue was arthroscopically confirmed presentation of the covering of implanted defects comprising cartilaginous tissue with good tissue integration with adjacent cartilage in one case at 12 months after the operation, in which its indication for a second-look arthroscopy was given by arthrofibrosis or meniscus-lesions (Fig. 5).

Histologic evaluation

Moreover, no complication was observed in the repaired tissue in other cases. Different characteristics of the osteochondral junction in normal and repair cartilage were revealed by HE and S-O stain. Histology of the biopsy specimens showed repaired tissue with cartilaginous tissue exhibiting positive S-O in one case and provided the best distinction between red- or pink-stained sulfated glycosaminoglycans and green-stained subchondral bone. The detailed observation showed that the superficial zone of the repaired tissue contained predominantly spindle-shaped fibroblast-like cells. By contrast, the majority of the deeper repair matrix in all the cases showed positive staining with safranin O-fast green and contained round-shaped cells in the lacuna, which suggested that the repaired tissue especially with regard to a hyaline cartilage-like matrix. Furthermore, the interface between the repair cartilage and subchondral bone exhibited a normal osteochondral junction. Thus, a zonal structure for the repair cartilage was confirmed in one of the cases. In contrast, as shown in the HE stain, the newly formed cartilage was naive chondrocytes in the shape of the round or oval, and the tidemark separating the cartilage and subchondral bone cannot be identified well in newly regenerated tissue. Given mature chondrocytes

looking flattered relative to more basal zones in the superficial area and more spheroidal in the transitional or middle zone[16], HE stains demonstrated oval or round-shaped cells with the phenotypic characteristics of native chondrocytes, while S-O stain readily discriminates cartilage from the fast green-stained bone, indicating that the cells were surrounded by sulfated proteoglycan-rich ECM typical of cartilage. Sections were immunostained for collagen type II and collagen type I, and observed by microscopy, and immunohistochemistry staining revealed type II collagen deposition on all type I scaffolds and minimal staining for type I collagen suggesting that this technology generated hyaline cartilage (Fig. 6).

Discussion

In this study, the efficacy of type I collagen-based MACT to mediate regenerative cartilage repair was demonstrated in a prospective clinical study with a 2-year follow-up. Positive clinical and morphologic outcomes were consistently evident across all 20 patients involved without any significant adverse events.

In the current study, the Knee injury and Osteoarthritis Outcome Score (KOOS) was used as the primary outcome. All five domains improved equally in all cases, and this improvement was sustained within the 2-year follow-up. The sports and recreation and quality were somewhat lower than for the other sections. This might be related to the fearful psychological factors after cartilage mechanical initial damage. Due to concerns regarding reinjury, patients felt hesitant to participate in the active exercise on a regular basis or other recommended moderate physical activity to facilitate rehabilitation after an operation, and this suggested that the doctor needed to supply the appropriate psychological counseling according to the psychological characteristics of patients to promote patients to recover to health earlier. However, this operation has achieved satisfactory results in patients in the second year after the operation. Our study showed good clinical efficacy with a large number of cartilage-like tissues filling the lesion, and this had also been evaluated in several studies. In a prospective clinical following 31 patients with MACI for 5 years, Ebert JR et al. [17] found good clinical outcomes, and all grafts were preserved on follow-up MRI except for two graft failure cases due to the complications. Moreover, Barié et al.[18] aimed to establish whether MACI or the second-generation ACI provided superior long-term outcomes in terms of patient satisfaction, clinical assessment, and MRI evaluation by following up 16 patients for an average of 9.6 years in a randomized clinical trial, and this study suggested that MACI method was equally effective treatments for isolated full-thickness articular cartilage lesions. The current study was different from the above-mentioned studies in that it had a shorter follow-up time of 24 months, and that the entire procedure was performed using the mini-open parapatellar arthrotomy. In parallel, arthroscopic exploration of nascent tissue formation was speculated to be the immature cartilage tissue accounting for the regenerated cartilage presenting with generalized tissue fragility, and the regenerated cartilage and subchondral bone were not fully integrated. This was consistent with the results of the quantitative MRI

T2-mapping according to the T2 values, which were still more than the surrounding cartilage. At the moment, we could only speculate that the postoperative recovery phase was more than 24 months.

Several reports involved another technique for MACI just as in the current study. Yoon et al.[19], in a prospective study, enrolling ten patients undergoing arthroscopic gel-type autologous chondrocyte implantation (GACI), manifested that GACI produced satisfactory clinical, radiologic, and histologic evaluation, which confirmed sufficient regeneration of hyaline-like cartilage that correlated improved symptoms. Since the GACI was infused in liquid form, it was applied to the dependent position of the lesion regardless of its geometry, and the implant could then spread by itself over the lesion site. Perhaps it could be a considerably simpler technique than other arthroscopic MACI methods. However, it required meticulous bleeding control for clear visualization of the lesion during the application of chondrocytes by using a suction syringe and cotton bud. The amount of handling and manipulation of the implant to prepare it for an arthroscopic procedure might lead to additional chondrocyte cell apoptosis[20]. The GACI was the lack of fixation causing the graft to fall off, and cell leakage and chondrocyte distribution were still an issue. On the contrary, because the implant in the current study was treated with a solid scaffold made of a kind of non-flowing gel, it was fixed in place with fibrin glue and no membrane cover, and MACT was performed as a mini- arthrotomy procedure for reimplantation. Furthermore, as the cases in this study, a small cadaveric study demonstrated that 16 times more viable cells remained on the membrane scaffold after implantation when the procedure was performed via a mini-open arthrotomy compared with arthroscopically[20].

MRI was widely performed to evaluate the quality of the repair cartilage while avoiding the potential sampling bias accompanying tissue biopsy, and its results confirmed the lesion filling with homogeneous tissue and a high integration ratio[21, 22]. The quantitative MRI was claimed to be currently the best tool to assess repair quality after implantation and before operation. However, the ability of quantitative T2 mapping was still in dispute, referring to providing a sufficiently detailed structural evaluation of regenerated cartilage considering the somewhat limited resolution of the currently available imaging technology[23]. Our results for MRI T2 mapping could not detect the morphologic differences between the superficial and mid to deep zone of the repaired tissue, and that could be detected from the histologic elevation. Thus, although invasive, the histologic assessment was likely still the most reliable method for detailed structural quality evaluation of cartilage repair.

Histologic evaluation was performed on one of the twenty patients at 12 months after the transplantation. Evaluation of the distribution of type II collagen showed hyaline-like cartilage in one patient who underwent second-look arthroscopy with biopsy. Similarly, Enea et al.[20] found hyaline cartilage, or a mixture of hyaline–fibrocartilage in 9 of 33 cases at a mean of 15 months after MACI. David et al. [6] revealed excellent histological results regarding the regeneration of hyaline articular cartilage in all patients. Although the current study included a smaller number of cases and follow-up time than these previous studies, its outcome was still comparable. Moreover, direct comparisons between the findings of the current study and these previous series may not be feasible, due to variations among studies in their patient demographics, follow-up duration, cell handling techniques, and histologic

grading systems. Alternatively, one biopsy showed an intensive expression of collagen II in the superficial zone of the biopsy with a slight decrease of intensity in the basal and middle of the specimen. Nonetheless, the clinical outcomes of this study showed bigger improvements compared to those of preoperation. However, the current study was still inferior to the above research. The reason for this might be the higher baseline scores and shorter follow-up time of the current study, and it caused a relatively narrow improvement when it was compared to the previous literature.

This study had several limitations. First, the procedure of this study was not compared to other operative techniques. Although performing reimplant steps of the procedure under mini-open and using the non-flowing gel scaffold might theoretically provide several advantages, further studies were required to compare operative time, postoperative rehabilitation, and clinical and radiologic outcomes with arthroscopic autologous chondrocytes combined with type I collagen scaffold. Second, since the sample size calculation was not performed with a certain clinical outcome measurement tool, an important drawback of this study was the small sample size. Third, the follow-up time was also relatively short for some evaluations. Clinical outcomes were followed for up to 5 years, and it was confirmed that improved clinical scores were maintained for a long period[24]. Lastly, histological evaluations were performed on just one patient at 12 months after implantation, and the number of histological evaluations was relatively small. Therefore, the current study was not able to assess implant integrity and any change in composition and distribution of type II collagen in the long-term, and it posed a significant challenge to get complete data to support our view.

Conclusions

Type I collagen-based MACT for the treatment of chondral or osteochondral lesions of the knee was a clinically effective treatment that yielded significant functional improvement and improvement in pain level, and this method presented histologic evidence of regenerating hyaline-like cartilage in the knee with articular cartilage defect by the biopsy in one case. On the contrary, the quantitative T2-mapping test showed that there was a difference between the regenerated cartilage and the surrounding hyaline cartilage.

Declarations

Disclosures and Acknowledgments

The authors disclose no conflict of interest in the writing of this article.

This study was supported by the Science and Technology Committee of Shanghai.

Authors' contributions

PLF designed the experiments and XL performed the experiments. JTQ helped XL perform some experiments and recorded experimental data. XL instructed SAL and YCC for statistical analysis. XL wrote

the paper and PLF made comments, suggested appropriate modifications, and made corrections. All authors have read and approved the final version of the manuscript submitted for publication.

Availability of data and materials

The experimental data will be available on request.

Ethics approval and consent to participate

Patient consent and approval from the Institutional Research Ethics Committee of the hospital were obtained to use these clinical materials for research purposes.

Consent for publication

Not applicable.

References

1. Carey JL, Remmers AE, Flanigan DC. Use of MACI (Autologous Cultured Chondrocytes on Porcine Collagen Membrane) in the United States: Preliminary Experience. *Orthop J Sports Med.* 2020,8(8):2325967120941816.
2. Krill M, Early N, Everhart JS, Flanigan DC. Autologous Chondrocyte Implantation (ACI) for Knee Cartilage Defects: A Review of Indications, Technique, and Outcomes. *JBJS Rev.* 2018,6(2):e5.
3. Solheim E, Hegna J, Inderhaug E. Long-Term Survival after Microfracture and Mosaicplasty for Knee Articular Cartilage Repair: A Comparative Study Between Two Treatments Cohorts. 2020,11(1):71-76.
4. Levine DW, Roaf PL, Duguay SJ. Characterized chondrocyte implantation results in better structural repair when treating symptomatic cartilage defects of the knee in a randomized controlled trial versus microfracture. *Am J Sports Med.* 2009,37(3):e3, author reply e4.
5. Christensen BB, Olesen ML, Hede KTC, Bergholt NL, Foldager CB, Lind M. Particulated Cartilage for Chondral and Osteochondral Repair: A Review. 2020:1947603520904757.
6. Grevenstein D, Mamilos A, Schmitt VH, Niedermair T, Wagner W, Kirkpatrick CJ, Brochhausen C. Excellent histological results in terms of articular cartilage regeneration after spheroid-based autologous chondrocyte implantation (ACI). *Knee Surg Sports Traumatol Arthrosc.* 2021,29(2):417-421.
7. Andriolo L, Reale D, Di Martino A, De Filippis R, Sessa A, Zaffagnini S, Filardo G. Long-term Results of Arthroscopic Matrix-Assisted Autologous Chondrocyte Transplantation: A Prospective Follow-up at 15 Years. *Am J Sports Med.* 2020,48(12):2994-3001.
8. Niemeyer P, Salzmann G, Feucht M, Pestka J, Porichis S, Ogon P, Sudkamp N, Schmal H. First-generation versus second-generation autologous chondrocyte implantation for treatment of cartilage defects of the knee: a matched-pair analysis on long-term clinical outcome. *Int Orthop.* 2014,38(10):2065-2070.

9. Ozturk E, Hobiger S, Despot-Slade E, Pichler M, Zenobi-Wong M. Hypoxia regulates RhoA and Wnt/beta-catenin signaling in a context-dependent way to control re-differentiation of chondrocytes. *Sci Rep.* 2017,7(1):9032.
10. Jimenez G, Lopez-Ruiz E, Kwiatkowski W, Montanez E, Arrebola F, Carrillo E, Gray PC, Izpisua Belmonte JC, Choe S, Peran M, Marchal JA. Activin A/BMP2 chimera AB235 drives efficient redifferentiation of long term cultured autologous chondrocytes. *Sci Rep.* 2015,5:16400.
11. Xu B, Fan D, Zhao Y, Li J, Wang Z, Wang J, Wang X, Guan Z, Niu B. Three-Dimensional Culture Promotes the Differentiation of Human Dental Pulp Mesenchymal Stem Cells Into Insulin-Producing Cells for Improving the Diabetes Therapy. *Front Pharmacol.* 2019,10:1576.
12. Gamez C, Schneider-Wald B, Schuette A, Mack M, Hauk L, Khan AUM, Gretz N, Stoffel M, Bieback K, Schwarz ML. Bioreactor for mobilization of mesenchymal stem/stromal cells into scaffolds under mechanical stimulation: Preliminary results. *PLoS One.* 2020,15(1):e0227553.
13. Ebert JR, Schneider A, Fallon M, Wood DJ, Janes GC. A Comparison of 2-Year Outcomes in Patients Undergoing Tibiofemoral or Patellofemoral Matrix-Induced Autologous Chondrocyte Implantation. *Am J Sports Med.* 2017,45(14):3243-3253.
14. Brittberg M. Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced autologous chondrocyte implantation procedure. *Am J Sports Med.* 2010,38(6):1259-1271.
15. Chen CH, Liu JM, Chua CK, Chou SM, Shyu VB, Chen JP. Cartilage Tissue Engineering with Silk Fibroin Scaffolds Fabricated by Indirect Additive Manufacturing Technology. *Materials (Basel).* 2014,7(3):2104-2119.
16. Baumann CA, Hinckel BB, Bozynski CC, Farr J. Articular Cartilage: Structure and Restoration. *Joint Preservation of the Knee.* Springer, 2019:3-24.
17. Ebert JR, Fallon M, Wood DJ, Janes GC. A Prospective Clinical and Radiological Evaluation at 5 Years After Arthroscopic Matrix-Induced Autologous Chondrocyte Implantation. *Am J Sports Med.* 2017,45(1):59-69.
18. Barie A, Kruck P, Sorbi R, Rehnitz C, Oberle D, Walker T, Zeifang F, Moradi B. Prospective Long-term Follow-up of Autologous Chondrocyte Implantation With Periosteum Versus Matrix-Associated Autologous Chondrocyte Implantation: A Randomized Clinical Trial. *Am J Sports Med.* 2020,48(9):2230-2241.
19. Yoon TH, Jung M, Choi CH, Kim HS, Lee YH, Choi YS, Kim SJ, Kim SH. Arthroscopic gel-type autologous chondrocyte implantation presents histologic evidence of regenerating hyaline-like cartilage in the knee with articular cartilage defect. *Knee Surg Sports Traumatol Arthrosc.* 2020,28(3):941-951.
20. 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.
21. Welsch GH, Zak L, Mamisch TC, Resinger C, Marlovits S, Trattnig S. Three-dimensional magnetic resonance observation of cartilage repair tissue (MOCART) score assessed with an isotropic three-

dimensional true fast imaging with steady-state precession sequence at 3.0 Tesla. *Invest Radiol.* 2009,44(9):603-612.

22. Mumme M, Barbero A, Miot S, Wixmerten A, Feliciano S, Wolf F, Asnaghi AM, Baumhoer D, Bieri O, Kretschmar M, Pagenstert G, Haug M, Schaefer DJ, Martin I, Jakob M. Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. 2016,388(10055):1985-1994.
23. Salzmann GM, Erdle B, Porichis S, Uhl M, Ghanem N, Schmal H, Kubosch D, Sudkamp NP, Niemeyer P. Long-term T2 and Qualitative MRI Morphology After First-Generation Knee Autologous Chondrocyte Implantation: Cartilage Ultrastructure Is Not Correlated to Clinical or Qualitative MRI Outcome. *Am J Sports Med.* 2014,42(8):1832-1840.
24. Ebert JR, Robertson WB, Woodhouse J, Fallon M, Zheng MH, Ackland T, Wood DJ. Clinical and magnetic resonance imaging-based outcomes to 5 years after matrix-induced autologous chondrocyte implantation to address articular cartilage defects in the knee. *Am J Sports Med.* 2011,39(4):753-763.

Figures

Figure 1

KOOS Score (Knee injury and Osteoarthritis Outcome Score) outcome after operation. Preop-preoperation, Pop-postoperation, mons-months, activities of daily living (ADL), sports and recreation (Sports&Rec), quality of life (QOL). aP <0.05 (vs preoperatively), bP <0.05 (vs 3 mons), cP <0.05 (vs 6 mons), dP <0.05 (vs 12 mons).

Figure 2

T2 mapping of cases after the operation. The chondral lesion treated with the implant developed an intensity similar to the surrounding normal cartilage over time. ROI-the region of interest. The minimum value of blue is 0 ms representing healthy cartilage, the maximum value of red is 100 ms representing damaged cartilage. PDW-proton density-weighted, SPAIR- spectral attenuated inversion recovery,mons-months.

Figure 3

MOCART Score after operation. Pop-postoperation,mons-months. aP <0.05 (vs pop 3mons), bP <0.05 (vs pop 6 mons), cP <0.05 (vs pop 12 mons)

Figure 4

Comparison T2 values of repaired site and surrounding normal cartilage after the operation. Preop-preoperation, Posop-postoperation, mons-months. aP <0.05 (vs preoperatively), bP <0.05 (vs 3 mons), cP <0.05 (vs 6 mons), dP <0.05 (vs 12 mons) eP p<0.05(vs normal cartilage)

A

B

Figure 5

Arthroscopic findings. A: Arthroscopic outcome before implantation, B: A second-look arthroscopy at 12 months. The lesion is covered with cartilage-like tissue after the matrix-associated autologous chondrocyte transplantation.

Figure 6

Histologic evaluation. The sample was taken from second-look arthroscopy performed at 12 months after the operation. A: Safranin O-fast green (S-O) stain for glycosaminoglycan distribution, B: hematoxylin and eosin (HE) stain to observe general cell morphology, C: immunohistochemically (IHC) with type-specific antibodies against collagen type II, D: immunohistochemically (IHC) with type-specific antibodies against collagen types I to evaluate collagen distribution of regenerated cartilage