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Review

A novel scaffold-free mesenchymal stem cell-derived tissue engineered construct for articular cartilage restoration - From basic to clinic

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ABSTRACT

Treatments for articular cartilage injuries are still challenging, due in part to its avascular and aneural surroundings. Since the first report of autologous chondrocyte implantation, cell-based therapies have been extensively studied with a variety of cell sources, including chondrocytes and mesenchymal stem/stromal cells (MSCs). Recently, MSC-based therapy has received considerable research attention because of the relative ease in handling for tissue harvest, and subsequent cell expansion and differentiation. Using such cells, we have originally developed a 3-dimensional scaffold-free tissue-engineered construct (TEC) through simple-cell culture methods and demonstrated its feasibility for cartilage repair and regeneration in the first-in-human clinical trial. This review summarizes our novel scaffold-free approaches to use MSC for the restoration of damaged articular cartilage, documenting the progression from basic to clinical studies.

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Abbreviations: ACI, autologous chondrocyte implantation; MSC, mesenchymal stem/stromal cell; TEC, tissue engineered construct; ECM, extracellular matrix; Asp-2p, ascorbic acid-2 phosphate; GAG, glycosaminoglycan; MRI, magnetic resonance imaging; KOOS, Knee injury and Osteoarthritis Outcome Score; MOCART, magnetic resonance observation of cartilage repair tissue; OA, osteoarthritis.

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1. Introduction

Articular cartilage injuries are common in patient populations treated in the field of orthopaedic surgery. In a prospective study of 1000 knee arthroscopies, focal chondral or osteochondral defects were found in 19% of the patients [1]. In these patients, 61% related their current knee problem to a previous trauma, and a concomitant meniscal or anterior cruciate ligament injury was found in 42% and 26%, respectively.

Articular cartilage does not usually heal spontaneously due in part to its avascular and aneural surroundings, as well as its relatively unique matrix organization arising during development and maturation [2]. Once damaged, articular cartilage injuries can progress to osteoarthritis (OA) because of the inability of chondral lesions to heal effectively. For patients with OA, they would be often forced to reduce their physical activity and undergo substantial modifications to their lifestyle. In addition, OA has a large social impact, and the average annual cost of OA for an individual is estimated to be between $700–$15,600 [3]. Therefore, a variety of approaches have been tested to improve cartilage healing outcomes, as well as prevent the development of OA over the past few decades [2,4].

Since the first results on autologous chondrocyte implantation (ACI) were published by Brittberg et al., in 1994 [5], cell-based therapies have been extensively studied with a variety of cell sources, including autologous chondrocytes and mesenchymal stem/stromal cells (MSCs). Chondrocyte-based therapies have provided favorable clinical results [6–9]. However, these procedures may have limitations including the sacrifice of undamaged cartilage within the same joint, as well as potential alterations associated with the in vitro expansion of the cells. Furthermore, due to the degenerative changes in cartilage that can accompany aging, the availability of cells may be limited in elderly individuals, both quantitatively and qualitatively [10]. In contrast, MSC-based therapies have become a focus to facilitate regenerative tissue repair, an approach which might be able to overcome such potential problems. MSCs have the capability to differentiate into a variety of connective tissue cells including bone, cartilage, tendon, muscle, and adipose tissue [11]. Additionally, these cells can be isolated from various tissues such as bone marrow, skeletal muscle, synovial membrane, adipose tissue, and umbilical cord blood [11–16], as well as synovial fluid [17]. MSCs isolated from synovium may be well suited for cell-based therapies for cartilage repair because of the relative ease of harvest and their strong capability for chondrogenic differentiation [13,14].

In addition to selection of an optimal cell source, effective local delivery of cells to chondral lesions could be another important issue for a successful cell-based therapy. Currently, 3-dimensional (3D) scaffolds, which are seeded with cells, is a widely utilized approach to enhance repair of these defects [18]. However, there are several issues associated with the long-term safety and efficacy of these materials, since such scaffolds are generally fabricated with synthetic polymers and/or biological materials [19]. Thus, such materials should ideally be excluded throughout the treatment procedure in order to reduce unknown risks, and in this regard, a scaffold-free cell delivery system would be an excellent alternative [20].

We have originally developed a novel scaffold-free 3D tissue-engineered construct (TEC) that is comprised of synovium-derived MSCs and extracellular matrices (ECMs) synthesized by the cells, and tested their feasibility for cartilage repair. Such a new, scaffold-less, MSC-based technique has gained attention as the next generation vehicle for cartilage repair [21]. In the present review, the suitability and effectiveness of the TEC methodology for cartilage repair and regeneration will be discussed from the basic characterization to the on-going clinical applications.

1.1. In vitro development of the TEC

When synovium-derived MSCs were cultured to confluence in a basic growth medium, they did not synthesize an abundant collagenous matrix. In contrast, in the presence of >0.1 mM ascorbic acid-2 phosphate (Asc-2P), collagen synthesis significantly increased with time in culture [19]. Subsequently, the cell-matrix complex cultured in Asc-2P became a stiff sheet-like structure comprised of MSCs and an ECM synthesized by the cells (Fig. 1A). After detachment from the culture vessel by gentle mechanical release, such a sheet immediately become a thick 3D tissue by active contracture (Fig. 1B). This contracted tissue was termed a tissue engineered construct (TEC) derived from MSCs. Immunohistochemical analysis showed that the TEC was rich in fibrillar collagen such as type I and III collagen [22]. In contrast, there was no detectable expression of type II collagen within the TEC. When such TECs were cultured in chondrogenic medium, synthesis of both glycosaminoglycan (GAG) and type II collagen were increased. Additionally, adhesion molecules such as fibronectin and vitronectin were also abundant in these TEC. It was found that these TEC are pliable and highly adherent to normal cartilage and therefore, suture-less implantation to damaged chondral surfaces is possible [23].

1.2. Preclinical animal study of the TEC in porcine chondral defect model

One of the crucial factors that may affect the results of cell-based therapies is the age of the donors and the recipients. While most studies have used autologous cell therapies, some have advocated using allogeneic circumstances, so the donor age and recipient age are relevant in both conditions.

Regarding the cell proliferation and differentiation capacities of MSCs, it is controversial as to whether they are age-dependent [24–27] or not [13,28–31]. In terms of the host tissue reaction, natural healing responses of osteochondral defects has been compared between immature and mature animals using rabbit models, and in this species, the studies demonstrated better healing responses in immature animals [32–35]. On the other hand, there have been no studies which compared the results of cell-based repair of chondral defects between immature and mature animal models. Regarding the use of a clinically relevant animal model for cartilage repair, it is difficult to create a chondral injury which does not breach the subchondral bone in small animals such as mice, rats, and rabbits due to the limited thickness of their articular cartilage, and thus, these conditions may not be as clinically relevant. In consideration of clinical relevance, it is preferable to utilize a large animal model to investigate the influence of skeletal maturity on the results of cell-based therapies to repair

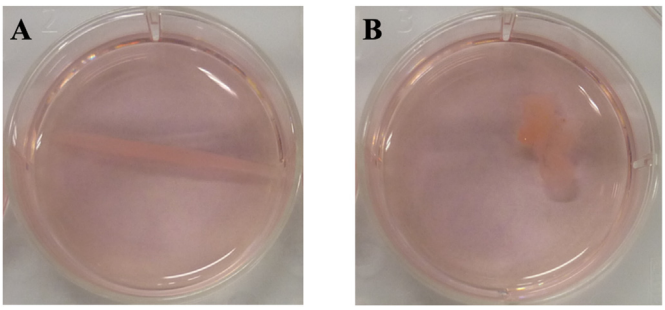

Fig. 1. Macroscopic view of the TEC. (A) TEC was partially detached from culture dish. (B) After detachment, TEC immediately become a thick 3D tissue by actively contracture. TEC, tissue-engineered construct.

chondral lesions. Therefore, in order to assess the efficacy of the TEC in an in vivo model, a porcine model was chosen as the physiology of the pig is similar to that of humans in many respects [36], and porcine articular cartilage of the knee is sufficiently thick as to allow creation of a chondral defect without damaging the subchondral bone.

Prior to an in vivo study, we compared the in vitro characteristics of cell proliferation and chondrogenic capacity in porcine MSCs isolated from skeletally immature animals (3–4 months of age) with mature animals (>1 year of age). Cell proliferation assays demonstrated that there were no significant differences between porcine synovial MSCs derived from immature and mature animals [23]. Similarly, there were no significant differences in chondrogenic capacities between MSCs isolated from immature and mature animals [23].

To test the feasibility of using the porcine TEC approach for a wide range of recipient ages to repair a chondral injury, immature as well as mature porcine chondral injury models were utilized in the preclinical studies. Full-thickness chondral defects of 8.5 mm diameter which did not breach the subchondral bone were created on the medial femoral condyle, and then the TECs were implanted into the defects in the immature and mature pigs. After implantation, the TEC firmly adhered to the injured joint surface without suturing. At 6 months post-implantation, regardless of starting age, untreated lesions exhibited no evidence for repair or only partial tissue coverage, while the defects treated with a TEC were covered with cartilaginous repair tissue. Histologically, the chondral lesions in the non-treatment control groups showed evidence of osteoarthritic changes, with loss of cartilage and destruction of subchondral bone in both skeletally immature and mature animals (Fig. 2A and B). Conversely, when treated with a TEC, the defects were filled with repair tissue exhibiting good integration to the adjacent cartilage and the restoration of a smooth surface (e.g. superficial zone of the cartilage), regardless of age at the time of implantation (Fig. 2C and D) [23]. Additionally, higher-magnification views indicated that the repair tissue treated with a TEC exhibited hyaline cartilage–like tissue characteristics with positive safranin O staining, regardless of the skeletal maturity of the animals (Fig. 2C and D). Following implantation, no histological findings were obtained that suggested either central necrosis of the implanted TEC or that an abnormal inflammatory macrophage and lymphocyte response consistent with some form of immunological rejection had occurred in this allogenic situation, regardless of the age of the recipient pigs. Also, it is important to note that following implantation, the TEC, which was initially type I collagen-rich, became devoid of type I collagen under the in vivo environment and became type II collagen-rich. Thus, the TEC was influenced significantly by the in vivo environment of the implantation site [22,37–39]. Moreover, the biomechanical properties of porcine chondral defects treated with a TEC approximated those of normal cartilage at 6 months post-implantation, regardless of age at the time of implantation [23].

1.3. First-in-human clinical trials using the TEC for repair of knee chondral lesions

Based on the encouraging results of the preclinical studies discussed above, we have now proceeded with clinical studies under

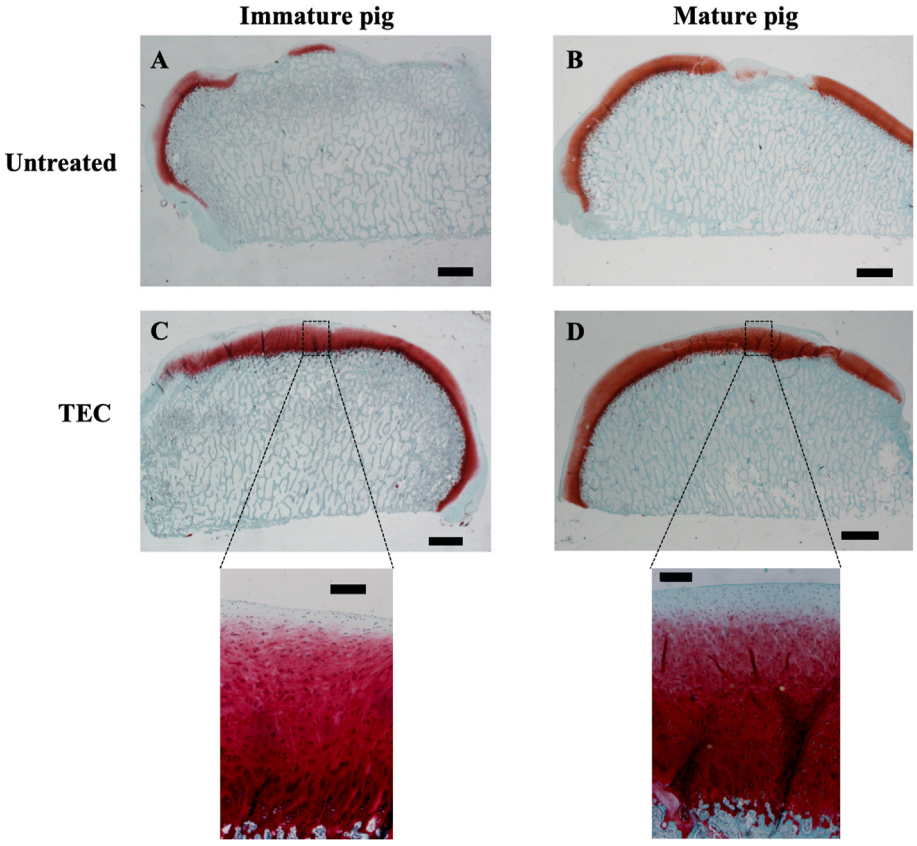

Fig. 2. Safranin O staining of untreated chondral lesions (A, B) or lesions treated with the TEC (C, D). (A, C) immature pig. (B, D) mature pig. Bar = 1 mm (upper images). Higher magnification view at the center area of the repaired tissue by the TEC were shown in the lower images. Bar = 200 μm. TEC, tissue-engineered construct. Quoted and modified with the publisher permission (License Number: 5754830663383) from Ref. No. 23 (Shimomura, K. et al., The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. Biomaterials, 2010. 31(31): p. 8004–8011. Copyright © 2010, © Elsevier Ltd. https://doi.org/10.1016/j.biomaterials.2010.07.017).

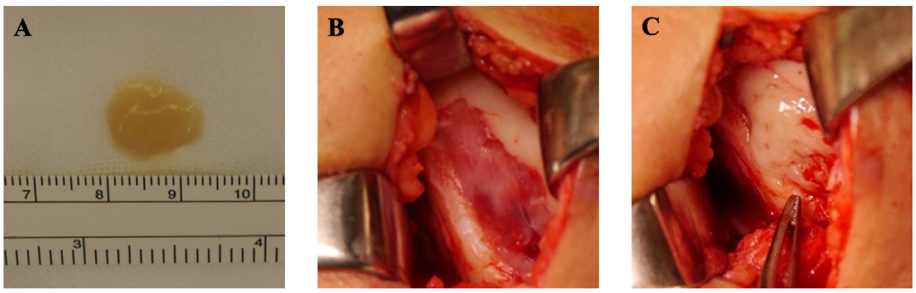

Fig. 3. Implantation of TEC generated from synovial mesenchymal stem cells. (A) Macroscopic view of TEC for implantation. (B) Cartilage defect on the medial femoral condyle. (C) Cartilage defect immediately after implantation with a TEC. TEC, tissue-engineered construct. Quoted and modified in accordance with the publisher reuse guideline from Ref. No. 41 (Shimomura, K. et al., First-in-Human Pilot Study of Implantation of a Scaffold-Free Tissue-Engineered Construct Generated From Autologous Synovial Mesenchymal Stem Cells for Repair of Knee Chondral Lesions. Am J Sports Med, 2018. 46(10): p. 2384–2393. Copyright © 2018, © SAGE Publications. DOI: 10.1177/0363546518781825).

the auspices of an approved first-in-human protocol [40]. Patients with symptomatic chondral lesions of the knee, and who meet the inclusion criteria (isolated chondral lesion ≦ 5 cm^2, 20–60 years of age, with normal alignment) have been enrolled [41].

Five patients (chondral defect, 1.5–3.0 cm^2; 4 men and 1 woman; age range, 28–46 years) participated in this study between February 2013 and April 2014, since the Ministry of Health, Labour and Welfare of Japan and the institutional ethical committee restricted approval for only 5 patients as an "early proof of concept" trial. Under general anesthesia, synovial membrane (>1g) was harvested from the knee joint, which was then subjected to the isolation and culture of MSC for their separation and expansion until passage 1 or 2. Following 4–6 weeks post-tissue harvest, the TECs were prepared for autologous implantation (Fig. 3A). By mini-arthrotomy, the chondral lesion was debrided so as to not breach the subchondral bone (Fig. 3B). Before implantation, the TEC was washed several times with sterile phosphate buffered saline to minimize serum-related protein contamination, followed by the adjustment of the TEC size to match that of the chondral defect. Implantation can be completed within 5–10 min, without any reinforcement for fixation (Fig. 3C). The knee is immobilized in a brace for 2 weeks followed by the initiation of range-of-motion exercises and muscle exercises. Full weight bearing is allowed 6–8 weeks after implantation surgery. Return to strenuous activity is allowed approximately 12 months following implantation. The primary outcome of this clinical study was the safety of the procedure. Secondary outcomes related to the efficacy of the procedure. Patients' satisfaction with the procedure was determined by self-assessment, and the morphologic quality of repair tissue was assessed using magnetic resonance imaging (MRI) out to 2 years

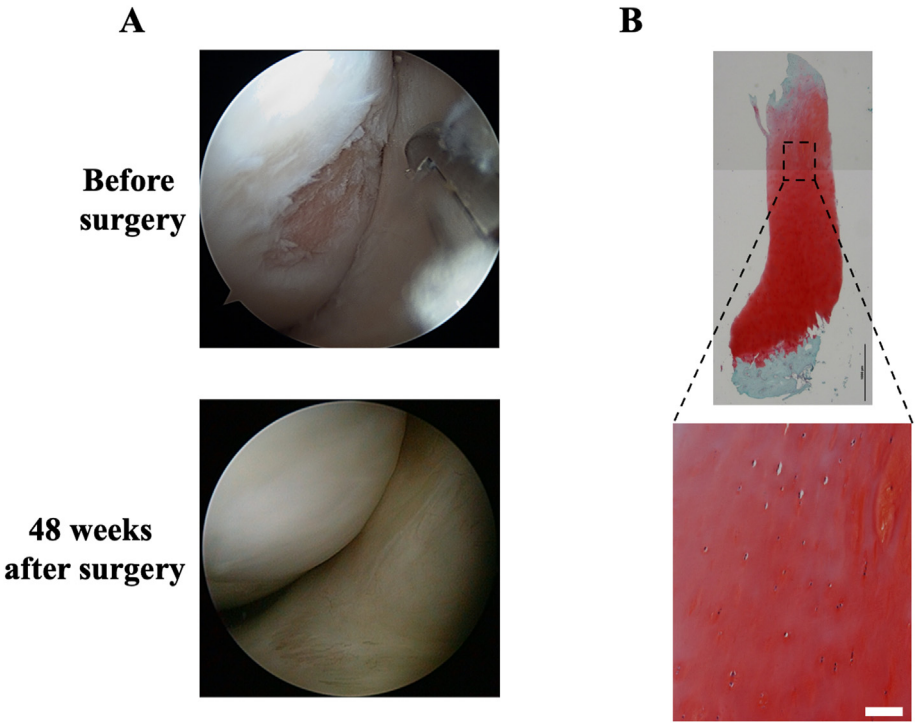

Fig. 4. Arthroscopic findings and histology of the biopsy specimens at 48 weeks post-implantation. (A) Arthroscopic image before implantation of the TEC and during a second look arthroscopy at 48 weeks postoperatively. The defects were covered with cartilage-like tissue at 48 weeks. (B) Safranin O staining of repair cartilage from biopsy specimens obtained at 48 weeks post-implantation. Repair tissue exhibited hyaline cartilage–like tissue characteristics with positive safranin O staining. Bar = 100 μm. TEC, tissue-engineered construct. Quoted and modified in accordance with the publisher reuse guideline from Ref. No. 41 (Shimomura, K. et al., First-in-Human Pilot Study of Implantation of a Scaffold-Free Tissue-Engineered Construct Generated From Autologous Synovial Mesenchymal Stem Cells for Repair of Knee Chondral Lesions. Am J Sports Med, 2018. 46(10): p. 2384–2393. Copyright © 2018, © SAGE Publications. DOI: 10.1177/0363546518781825).

after surgery, as well as the macroscopic and histologic analyses of repair tissue at 48 weeks. No adverse events were recorded during the two years post-surgery. Self-assessed clinical scores for pain, symptoms, activities of daily living, sports activity, and quality of life were significantly improved at 2 years after surgery. Secure defect filling was confirmed by second look arthroscopy (Fig. 4A) and MRI in all cases. Histology of biopsy specimens indicated repair tissue approaching the composition and structure of hyaline cartilage (Fig. 4B). Thus, these results provided evidence for the safety and efficacy of a scaffold-free TEC derived from autologous synovial MSCs to facilitate cartilage repair. With a simple and rapid implantation procedure, the TEC approach could provide a novel treatment option with high chondrogenic capacity, with potential medical cost reductions and good patient satisfaction.

1.4. Midterm outcomes after implantation of the TEC for repair of knee chondral lesions

As mentioned above, the safety and efficacy of TECs for cartilage repair at 2 years post-implantation was documented [41]. We further evaluated the clinical outcomes and MRI findings at 5 years post-implantation in the same cohort to assess the continued efficacy of the TEC treatment [42]. All clinical scores, including the visual analog scale for pain, Lysholm score, Tegner score, and Knee injury and Osteoarthritis Outcome Score (KOOS), were significantly improved from the preoperative evaluation to the 2- and 5-year follow-ups and the results were stable over time (Fig. 5A–E). The MRI scan evaluation showed cartilage defects filled with newly generated tissues with good tissue integration to adjacent host

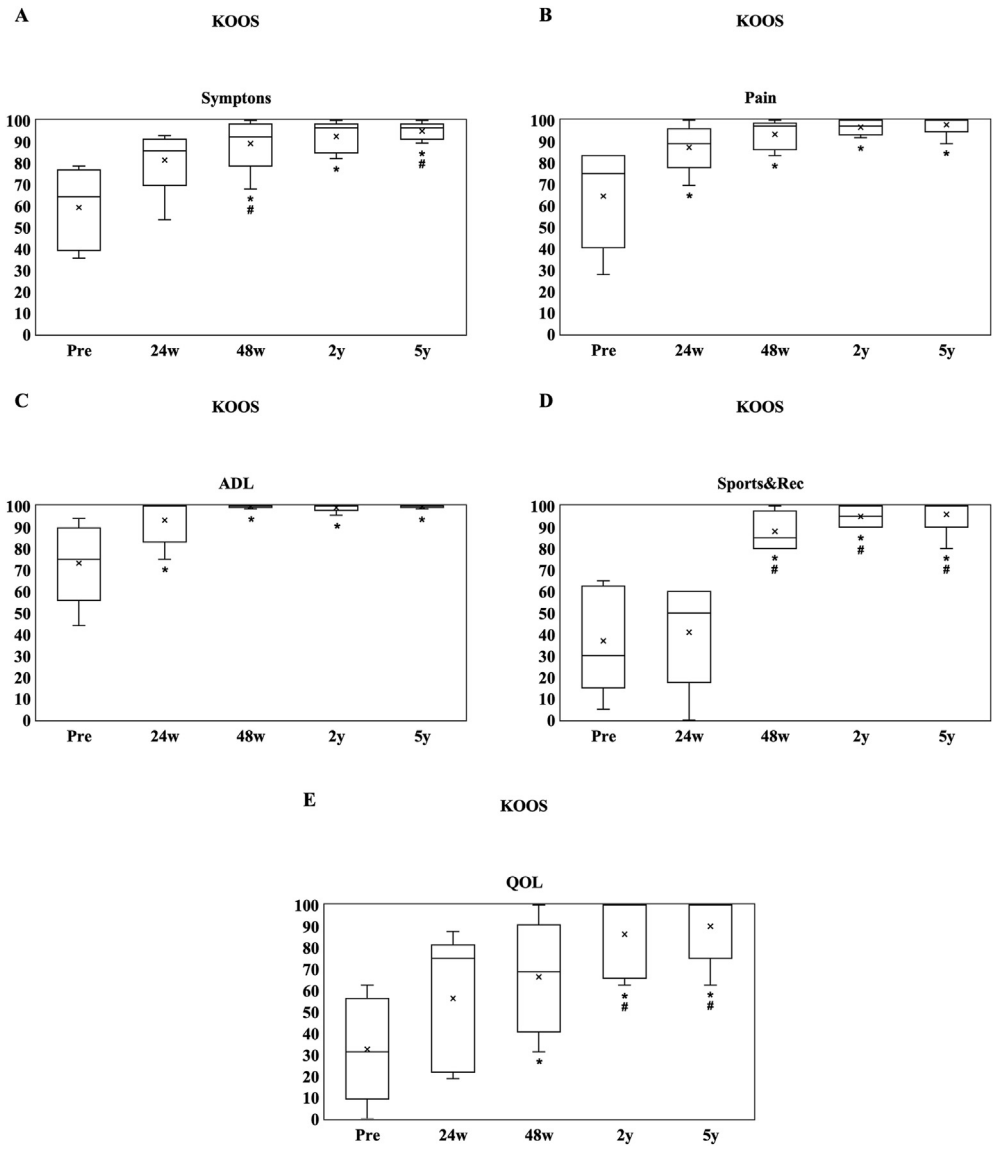

Fig. 5. Patient-reported outcome measures from preoperatively to 5-year follow-up: KOOS subscales (A) Symptoms, (B) Pain, (C) ADL, (D) Sports & Rec, and (E) QOL. Clinical improvements from baseline to 2 years were maintained stably up to 5 years. The × within the box indicates the mean, the horizontal line indicates the median, the top and bottom of the box indicate the interquartile range, and the whiskers indicate the range. Statistically significant differences: *versus preoperatively and #versus 24 weeks postoperatively ($P < 0.05$). ADL, activities of daily living; KOOS, Knee injury and Osteoarthritis Outcome Score; pre, preoperatively; QOL, quality of life; Sports & Rec, Sports and Recreational Activities; TEC, tissue-engineered construct. Quoted and modified in accordance with the publisher reuse guideline from Ref. No. 42 (Shimomura, K. et al., Five-Year Outcomes After Implantation of a Scaffold-Free Tissue-Engineered Construct Generated From Autologous Synovial Mesenchymal Stromal Cells for Repair of Knee Chondral Lesions. Orthop J Sports Med, 2023. 11(8): p. 23259671231189474. Copyright © 2023, © SAGE Publications. DOI: 10.1177/23259671231189474).

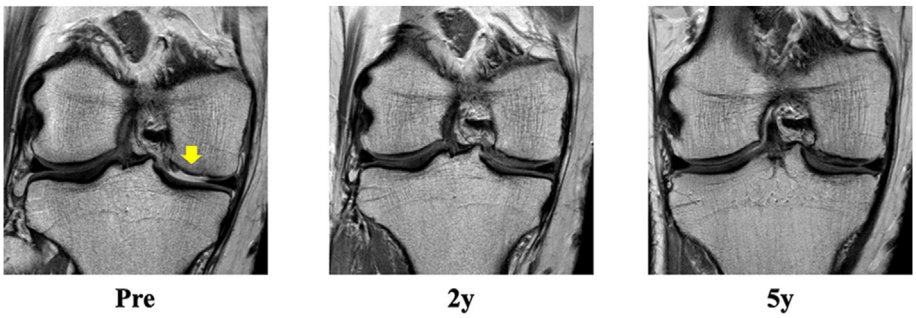

Fig. 6. Proton density-weighted MRI scans of the injured cartilage sites (yellow arrow) preoperatively, and at 2 and 5 years postoperatively. MRI, magnetic resonance imaging. Quoted and modified in accordance with the publisher reuse guideline from Ref. No. 42 (Shimomura, K. et al., Five-Year Outcomes After Implantation of a Scaffold-Free Tissue-Engineered Construct Generated From Autologous Synovial Mesenchymal Stromal Cells for Repair of Knee Chondral Lesions. Orthop J Sports Med, 2023. 11(8): p. 23259671231189474. Copyright © 2023, © SAGE Publications. DOI: 10.1177/23259671231189474).

cartilage over time (Fig. 6). The cartilage thickness and surface smoothness of the repair cartilage were maintained up to 5 years postoperatively. The magnetic resonance observation of cartilage repair tissue (MOCART) 2.0 Knee Scores [43] remained high at 5 years. Thus, these results highlight the efficacy and feasibility of the TEC for regenerative cartilage repair via a suture-less and simple implantation procedure, showing good clinical outcomes and MRI findings with stable results at midterm follow-up. Thus, in this pilot clinical trial, the patient outcomes mirrored the findings from the many of the preclinical large animal studies.

1.5. Future perspective and conclusion

The present review has integrated the findings obtained over the past several years demonstrating the feasibility of using a unique scaffold-free TEC generated from synovial MSCs for effective cell-based cartilage repair. Further, a phase III multicenter randomized controlled clinical trial with more patient enrollment to further evaluate the TEC approach versus microfracture was completed and the results are currently undergoing analysis as of April 2024 (JRCT ID: jRCT1080223548, TWOCELLS, Co., Ltd.). The results of this randomized controlled trial will further define the significance of this unique MSC-based therapy compared to another currently available treatment option (microfracture). Subsequent regulatory approval of the TEC product for the repair of chondral defects could potentially prevent the development of OA by early treatment of individuals suffering an injury to their articular cartilage.

As addressed in our earlier studies [19,23], there are several advantages to utilizing the TEC approach for cartilage repair. The TEC is generated through the simple and rapid scaffold-free manufacturing process with autologous synovium-derived MSCs, as compared with other cartilage tissue-engineering approaches [44–46]. Also, the TEC develops without any exogenous scaffold and thus, implantation of these TECs would have minimal risk of potential side effects induced by artificial or extrinsic biological materials contained in a scaffold. Moreover, the TEC is a soft spherical body with plasticity and adhesiveness to the cartilaginous matrix [22,47]. Such material properties would be advantageous, as they enable the ready matching to the needed shape and size for the repair of a chondral defect and allow for the rapid suture-less implantation by minimally invasive surgery. Thus, such TEC approaches could provide a novel treatment option with high chondrogenic capacity, safety, and lower cost.

As mentioned above, cartilage injuries might become curable with currently available cell-based therapies including the TEC approach. However, the current cell-based therapies can target on the patients with cartilage injury, whereas no effective treatments for those of OA have been developed. Thus, the bigger clinical problem is related to the large patient population with the currently incurable OA. As the incidence of OA is much higher than that for isolated chondral injuries [1,48,49], such OA patient population will require new interventions that can address the OA-joint environments [50]. Therefore, development of novel therapeutic methods for osteochondral repair are also urgently needed. As such lesions very often involve subchondral bone damage, it is important to also consider subchondral bone regeneration in addition to cartilage. Recently, we have combined the scaffold-free MSC-based TECs with an artificial bone block to fabricate a biphasic osteochondral implant and demonstrated the feasibility of using such constructs for osteochondral repair in a rabbit study [51,52]. Therefore, the combined TEC-artificial bone construct as another potential viable option for TEC application, could also be considered a promising MSC-based bio-implant to repair osteochondral lesions in the near future. However, as OA is considered a disease with inflammatory aspects [50], some additional modifications to the environment of the joint may be required to optimize the effectiveness of the TEC intervention to repair and regenerate the cartilage damage [53].

Additionally, being an initial type I collagen rich matrix, the TEC could be potentially suitable for augmenting repair of compromised skin, or enhancing the repair of ligaments or tendons, which are also type I collagen rich. Since the TEC also has chondrogenic, osteogenic or adipogenic differentiation capacity, the TEC could potentially be used for other applications. While still at the stage of preclinical animal studies, the feasibility of TEC use for the repair of growth plate [54], meniscus [55] and intervertebral disc [56] has been demonstrated. Moreover, as TECs could be developed from MSCs derived from other tissues, such as adipose tissue, which is an abundant source of MSC [57,58], they could be readily obtained without entering the joint and thus avoid any potential morbidity associated with removal of synovium. Therefore, future tissue engineering using the TEC technology could provide a variety of therapeutic interventions in regenerative medicine for a number of tissue applications using MSC from different sources to address the need for repair and regeneration of several musculoskeletal injuries affecting so many individuals.

Acknowledgments

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