Review Article

The comparison and analysis among clinical studies of tissue engineering in knee cartilage defects

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ABSTRACT

Treatment of cartilage damage is not satisfactory mainly due to cartilage nature that is avascular, so that its nutrition only depends on the diffusion process, which does not support regeneration. One of the new approaches for regeneration of injured cartilage that have a future is tissue engineering. There have been many clinical applications of tissue engineering in knee cartilage defects with varying results that it is not known, which method provides the best result. Therefore, this review highlighted the clinical applications of various tissue engineering methods in knee cartilage defects and did comparison of the various procedures. Our result showed that the best healing results was tissue engineering using GelrinC, and the method that had the lowest side effects was tissue engineering using hydrogel-based autologous chondrocyte transplantation.

***Keywords*:** Osteoarthritis, scaffold, hydrogel, chondrocytes

INTRODUCTION

One of the largest joints in the body, the knee, is made up of three bones: the femur, tibia, and patella, which are kept together by a complex network of ligaments, cartilage, tendons, and muscles [1]. The knee is an important structure that is

responsible for the movement of the lower leg and supports most of the body's weight, as well as various movements or activities that are load-bearing, such as walking, running, jumping, and lifting loads. Therefore, it is not surprising that the knee is very susceptible to injury. Knee injuries can also occur as a result of sports or recreational activities, falls, or simply normal wear and tear. Most minor injuries, such as cuts and bruises, can heal on their own, but other injuries can progress to chronic problems that impair knee function. Joint cartilage is a connective tissue that is responsible for compressive force resistance, load distribution, and together with synovial fluid allows frictionless movement of the bone surfaces that make up the joint components. Damage to the knee cartilage is an interesting topic in the increasing life expectancy of humans. However, like a double-edged sword, the longer a person live, the risk of degenerative diseases looming over his/her life. Many cases of knee osteoarthritis are the main manifestation of knee cartilage damage [2]. Although the real frequency of cartilage lesions is unknown, numerous studies show that 60-66 percent of knees undergoing arthroscopy have articular abnormalities. As a result, about 900,000 Americans are affected by knee cartilage injuries each year, resulting in over 200,000 surgical procedures [3].

For more than 400 years the treatment of cartilage damage has not achieved satisfactory results. Poor regeneration due to its avascular nature, minimal cells, without a basement membrane, and nerve innervation so that its nutrition only depends on the diffusion process [4]. This is increasingly challenging for orthopedic experts in the race to find a comprehensive therapeutic approach [2]. Symptomatic relieves may be offered by anti-inflammatory agents, and some herbal substances may offer beneficial effect [5,6], but those substances are not yet readily available. Along with the development of science and technology, Tissue Engineering (TE) has become an approach that has a future. Damaged tissues need blood vessels to be repaired, and vascular tissue engineering has progressed recently [7]. However, healthy articular cartilage should be avascular, thus vascular tissue engineering is not appropriate. In addition, the use of TE in the repair of knee injuries has also progressed. In the past decade, tissue engineering have incorporated cell and gene activating agents, signaling molecules, or growth factors into scaffolds [8]. Cells residing in the scaffold can induce cell adhesion,

differentiation, proliferation, and subsequent tissue regeneration for better healing [9].

So far, there have been many clinical applications of TE in knee cartilage defects with varying results. Some general and clinical application reviews concerning cartilage TE exist, but those reviews did not compare and conclude, which method was the best [10-14]. Moreover, two reviews were conducted thirteen years ago [11,13], and a review discussed the possibility of 3D bio-printing that had not enter human clinical application. Meanwhile, it is not known, which method in human studies provides the best result. Therefore, the aim of this review was to summarize the clinical applications of TE in knee cartilage defects in patients and to compare and analyze, which procedure is the best in providing healing for knee cartilage defects. This comparison and analysis between clinical studies is the novelty of this review. For this purpose we discussed the treatments of knee cartilage defects, various methods of TE to heal knee cartilage defects, comparison between various methods of tissue engineering and future directions.

Treatments of knee cartilage defects

Various studies related to knee cartilage defects have been carried out, while at the same time the treatment of knee cartilage defects has also progressed. Several factors should be considered when deciding which method of therapy to choose, such as the location of the lesion and the size of the defect (which can be determined by Magnetic Resonance Imaging/MRI), the age and activity level of the patient, which can be divided into two categories: a) young patients (< 40 years old) or patients with high activity and b) older patients (40–50 years) or patients with low activity level [15].

Tissue engineering provides an alternative to organ transplantation. This minimizes the rate of invasive surgery, but there is always the possibility of immunological rejection or failure to maintain function. The risk of failure can be reduced by engineering the tissue as closely as possible to the natural organ. To achieve this, there are several factors in the biological system that must be considered, namely (a) biomaterial factors that can cause non-specific biological effects and cause changes in the local mechanical environment, (b) biomaterial design to minimize immune responses that can cause inflammation and rejection, (c) biomaterial design to create suitable substrates in the process of cell survival and differentiation of implanted cells, and (d) the importance of maintaining a balance of the

microenvironment of the host and tissue replacement to maintain proper cell function towards repair and regeneration [2]. The development of optimal tissue engineering is carried out through a combination of factors described in the tissue engineering triad, namely the matrix (scaffold), signal, and cell [8].

Various methods of tissue engineering of knee cartilage

Injury to the knee cartilage can lead to cartilage degeneration, which can eventually lead to joint inflammation. Therefore, it is important to treat knee cartilage defects to stop or slow the progression of degeneration and inflammation. The current alternative to surgical method for cartilage tissue repair is a tissue engineering approach. Tissue engineering opens new avenues for knee cartilage regeneration [16]. Knee cartilage tissue engineering can use various methods, namely: tissue engineering using osteochondral biomimetic scaffold, or Autologous Chondrocyte Implantation (ACI) and porcine collagen bilayer membrane, or biodegradable biphasic osteochondral construct and minced autologus cartilage, or collagen-covered microfracture and bone marrow concentrate, or cartilage-like tissue implants, or MSC and fibrin glue, or osteochondral autograft transplantation, or

3D-pellet-type autologous chondrocyte, or hydrogel (Table 1) [17-28].

Tissue engineering using osteochondral biomimetic scaffold

Twenty-seven patients presenting with symptomatic Osteochondritis Dissecans (OCD) were treated with osteochondral biomimetic scaffold transplantation, which had a porous three-layer 3-D composite structure that can simulate a whole osteochondral architecture. This scaffold has a cartilage-like layer, which is composed of type I collagen with a smooth surface. The intermediate layer is made up of type I collagen (60 %) and hydroxyapatite (40 %), whereas the lower layer is made up of type I collagen (30 %) and hydroxyapatite (70 %). The use of osteochondral biomimetic scaffolds was reported to cause mild side effects after scaffold implantation, namely 2 patients experienced fever during the first week and 3 patients experienced joint stiffness. However, the final clinical evaluation gave good results. From the baseline evaluation through the 1 and 2 year follow-up points, all clinical ratings showed statistically significant improvements. Complete cartilage filling was seen in 72 percent of the lesions after a 2-year MRI, complete graft integration was seen in 83 percent, intact tissue surface repair in 56 percent, and homogeneous tissue structure repair in

39 percent. The simplicity of the surgical procedure and the plasticity of the graft allow the use of this scaffold to be applied also to large osteochondral lesions. The addition of other factors, such as cells or bioactive molecules can be considered to improve the function of the scaffold biomaterial [17].

The same thing was done by Kon et al. who also used osteochondral biomimetic scaffold transplantation with a porous 3-D composite three-layer structure in 79 patients with knee articular chondral lesions and osteochondritis dissecans. The results showed a significant increase in International Knee Documentation Committee (IKDC), graded activity based on work and sports activities (Tegner), and Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores from pre-treatment to 1-year and 2-year follow-up periods. The results of the final follow-up of MRI evaluation showed complete cartilage filling in 62.2 %, complete graft integration in 86.7 %, intact repaired tissue surface in 71.1 %, and tissue structural homogeneity in 48.9 % of patients. Swelling of the knee in the early postoperative period was experienced by 17 patients (21.5 %), and joint stiffness was seen by 9 patients (11 %). The authors stated that this biomimetic scaffold was effective for treating chondral and osteochondral knee defects despite a brief period of follow-up, as shown in a large population [18].

TE using ACI and porcine collagen bilayer membrane compared to periosteum

A retrospective study was conducted in England, which enrolled 88 patients who had ACI treatment for cartilage knee defect. In the study, 33 patients received ACI and Chondrogide (ACI-C) and 55 patients received ACI and Periosteum (ACI-P). Chondrogide is a commercially available bilayer membrane with a compact smooth surface that prevents cells from sliding through the membrane and a layer of porous collagen fibers that promote cell invasion and attachment, such as described by the manufacturer. The Lysholm score was used to assess the patients. The score is a 100-point system for evaluating a patient's knee symptoms, which include mechanical locking, instability, discomfort, swelling, stair climbing, and squatting. The Lysholm scores of patients treated with ACI-C were significantly lower before surgery and at biopsy than patients with ACI-P, but at the last follow-up, there was no significant difference in scores. In addition, Osscore was also used; it is a semiquantitative scoring system (total score of 10) that assesses several

parameters, including tissue morphology, metachromacy matrix, cell clusters, surface architecture, basic integration, calcification, and vascularization. The results of ACI-C biopsy showed a higher Osscore value, where the presence of hyaline cartilage and hyaline-fibrocartilage mixture was more than ACI-P. Moreover, in term of the proportion of fibrocartilage tissue repair, patients with ACI-P were significantly greater than patients with ACI-C, i.e. ACI-P was approximately 70 % and ACI-C was 40 %. In contrast to cellular morphology scores, ACI-C patients had considerably higher cellular morphology ratings than ACI-P patients. There was no significant difference in overall histology scores between the two groups. From the biopsy results, patients with ACI-C had a higher frequency of normal or moderate surface architecture (±80 %) than those with ACI-P (±50 %). Hyaline cartilage was found in 93 % of ACI-C samples and 85 % of ACI-P samples, which stained positive for collagen types I and II [19].

This study demonstrated that the cellular morphology and surface architecture of healing tissue, the morphology of the hyaline cartilage, and staining of type II collagen of ACI-C when compared to ACI-P treatment was much better. Unfortunately, the authors did not assess side effects experienced by patients, so it cannot be ascertained whether this tissue engineering was without side effects [19].

TE using biodegradable biphasic osteochondral construct and minced autologus cartilage

Keeping with the concept of ACI, Chiang et al carried out a modification, namely a biodegradable biphasic osteochondral construct in combination with minced autologous cartilage as a source of chondrocytes. This modification was made to avoid cell culture-expansion. Ten patients were treated with implantation of a biphasic osteochondral construct and successfully completed a two-year follow-up period. During the postoperative phase, there were no complications such as infection or significant hemarthrosis, and all wounds healed well one week after surgery. Second view arthroscopy revealed that the transplanted location was entirely covered with cartilage tissue with a smooth flat surface that blended well with the adjacent native cartilage without gaps in seven patients. The cartilage regeneration within the lesion was incomplete in the other three individuals, whose cartilage was mixed with coarse fibrous tissue and pits revealing suchondral bone. Histological examination showed cartilage tissue regeneration without any remaining biomaterial in the chondral phase. These

clinical results showed that healing occurs in 70% of patients and did not cause serious side effects after implantation so that biphasic osteochondral construct was thought to be safe for therapeutic use. The structure was securely fastened without the use of any additional fixation. This single-stage implantation is present as a solution due to graft source restrictions and donor site morbidity [20].

TE using collagen-covered microfracture and bone marrow concentrate

The Collagen-Covered Microfracture and Bone Marrow Concentrate (C-CMBMC) were used to treat nine individuals with symptomatic chondral lesions of the knee. This method is a technique that adopts a collagen membrane scaffold combined with microfractures [21].

Enea *et al.* who treated patients with C-CMBMC reported that one patient had failure due to persistent effusion, and one patient had worsening of pain until the last follow-up. According to the arthroscopic evaluation, near normal (grade 2) was assigned to four patients, with a median overall rating of 9.5. (range 9-11). The results of histological evaluation of those four patients showed that a hyaline-like matrix was found in 1 patient, fibrocartilage was found in 2 patients, and mixed hyaline/fibrocartilage was found in 1 patient. The collagen membrane was also discovered to be totally reabsorbed [21].

The authors concluded that the C-CMBMC approach was safe and successful in alleviating the symptoms of patients with isolated condylar cartilage lesions of the knee, and had the ability to promote hyaline-like cartilage repair, with a median follow-up of 29 months. However, looking at the almost normal healing rate, it was only four out of nine (44 %) and the histological evaluation also did not show uniform results [21].

TE using cartilage-like tissue implants

Tissue-engineered cartilage-like tissue implants were implanted in 72 patients with full-thickness knee cartilage defects. To create tissue engineered cartilage-like tissue, autologous chondrocytes were cultured in atelocollagen gel for 3-4 weeks. After culture, this technique produced a strong gel-like material in which the chondrocyte phenotype was preserved and an extracellular matrix was created [22].

A moderately long follow-up period (5–11 years, with an average of 8.0 years) of tissue engineered cartilage-like tissue implantation treatments resulted in the following clinical outcomes: arthroscopic results were normal or near normal two years after implantation (87,7 %) and graft stiffness nearly matched that of the

surrounding normal cartilage. The overall assessment of improved tissue based on the ICRS Visual Assessment Scale II (ICRS II) was 70.4+20.8 %. Mean MOCART scores continued to improve over the 1, 2, and 5-year follow-up periods. Moreover, the healing results according to arthroscopic findings were considered normal or almost normal, which occurred in 87.7 % of patients. However, the frequency of graft-related complications was assessed as high (30 %), including partial periosteal detachment, hypertrophy, ossification, and graft failure. The results of the histological evaluation showed that the regenerated tissue was not true hyaline cartilage. Nonetheless, the authors stated that tissue-engineered cartilage-like tissue implantation for knee cartilage defects with a median of 8.0 years postoperatively, was safe and effective [22].

TE using MSC and fibrin glue

Kim *et al.* conducted a prospective cohort study in which 20 patients with cartilage lesions in the knee were given Mesenchymal Stem Cell (MSC) implantation with fibrin glue as a scaffold for cartilage regeneration. After arthroscopic fluid was extracted, thrombin-fibrinogen cell suspension (MSC combined with fibrin glue) was implanted under arthroscopy guidance. Fibrin glue could boost the proliferation and expression of MSC genes, preserved survival and paracrine function, as well as enhanced the therapeutic efficacy of adipose-derived MSCs for cartilage repair [23].

In this study, the clinical results of MSC implantation with fibrin glue showed that there was a significant change in lesion grade at the final follow-up (mean 24.2 months) compared to the condition before surgery. According to the size of cartilage loss area, which was assessed by MRI, before surgery there were 21 lesions (87.5 %) of grade 2 or 3 and at follow-up only 5 lesions (20.8 %) were grade 2 or 3. According to the percentage of entire cartilage thickness loss, which was assessed by MRI, before surgery there were 23 lesions (95.9 %) of grade 2 or 3, while only 5 lesions (20.8 %) were grade 2 or 3 on follow-up. These results revealed that the quality of the repaired cartilage was increased, which was indicated by the rate of lesion healing and cartilage repair that reached 79.2 %. Furthermore, a strong correlation between MOCART score and clinical outcome was discovered ($p<0.001$). The authors believe that fibrin glue promotes cell survival, proliferation, differentiation, and matrix production, resulting in cartilage repair in osteoarthritis knees. However, the author did not assess

the side effects of this tissue engineering [23].

TE using osteochondral autograft transplantation

A study enrolled 112 patients with Focal Chronic Non-traumatic Osteochondral lesions (FCNO) of the knee who received osteochondral Autograft Transplantation (OAT). In OAT, the cartilage defect is filled with an osteochondral autograft with a higher amount of hyaline cartilage, compared to the Autologous Chondrocyte Implantation (ACI) technique [24].

The results of clinical and radiological examination of the study showed no severe complications that were found in the patients. Patients with FCNO lesions showed significantly better overall clinical outcomes after OAT. Clinical improvement was not limited to younger patients, but also in older patients where the effect was substantial and significant. Both quality of life, which was represented by Western Ontario and McMaster Universities Osteoarthritis (WOMAC) scores, and pain scores, which was represented by Visual Analog Scale (VAS) scores, were significantly lower postoperatively than before OAT. Clinical improvement was marked by a decrease in WOMAC and VAS scores after OAT surgery, which means an improved quality of life and reduced pain levels. The clinical evaluations that were carried out in this study were considered limited because they only produced VAS and WOMAC scores, and did not explicitly show the percentage of cure so that the effectiveness of the use of tissue engineering could not be assessed [24].

TE using 3D-pellet-type autologous chondrocyte implantation

Yoon *et al.* restored full-thickness cartilage defects in the knees of seven patients with Costal Chondrocyte-derived Pellet-type Autologous Chondrocyte Implantation (CCP-ACI). This scaffold (CartiLife), is a small pellet type ACI that is made from the patient's own chondrocytes from the costal cartilage, followed by expansion culture and Three-Dimensional (3D) pellet culture. The costal cartilage was chosen because it was judged to have a significant similarity with the articular cartilage. Costal cartilage chondrocytes are comparable to articular cartilage chondrocytes in terms of expansion ability and capacity, as well as the potential to form hyaline-like cartilage tissue. In this study, the clinical results showed that over a 5-year follow-up period, CCP-ACI was linked to improved clinical score. Increases in Tegner, Lysholm, and IKDC scores were found at 1-year, 2-year, and 5-year follow-up visits. The MRI results showed an increase in the average MOCART score at 3 follow-up visits,

namely 44.17±14.97, 51.67±14.02, and 55.0±17.32, while none of the patients showed worsening of filling defects over time. Complete filling of cartilage defects at 1 year were found in 2 patients and at 5 years were found in 4 patients, this result showed that the patient's healing rate reached 57.14 %. Adhesions were not discovered before surgery or during the 5-year follow-up period. In side effect assessments there were no signs of immune reactions in any of the participants, but procedural pain that was associated with costal cartilage biopsies and CCP-ACI transplanting was the most common side effect. All side effects were mild and resolved without sequelae or complications. There were no specific adverse reactions, such as immunological reactions, osteogenesis, or cancer, over the 5-year follow-up period [25].

According to the authors, CCP-ACI is a safe and effective treatment for defect of articular cartilage, in terms of symptom relief, knee function, and structural regeneration, although the success rate is not very high (57.14 %). A review by Owida [10] commented that the use of autologous chondrocytes was advantageous compared to other type of cells, including Mesenchymal Stem Cells (MSCs). Unfortunately, the number of samples is very small and limited to young patients (19 years), so it is not known how will be the result in older patients (>40 years) [25]. Regarding the use of MSCs, a review by Francis *et al.* [12] discussed the disadvantage of MSC use in *in vivo* conditions, which posed obstacles in providing differentiation cues into chondrocytes, as adult native synovium would not provide necessary growth factors for chondrocyte differentiation.

Tissue engineering using hydrogel

Trattnig *et al.* study, which enrolled 21 patients with 1-2 symptomatic lesions of femoral condyle, used GelrinC implantation as a treatment. GelrinC is a revolutionary cell-free biosynthetic hydrogel implant that has been created to facilitate consistent and successful cartilage regeneration in a one-step technique that does not require autologous or allogeneic cells. GelrinC is applied as a liquid into the defect so that it can completely fill it. GelrinC is turned into a soft elastomeric implant after 90 seconds of UVA radiation. These implants operate as scaffolds by progressively dissolving over time, allowing new cartilage to grow in its place and being absorbed fully in 6-12 months. This study reported the outcome of treatment in patients with GelrinC implants that showed MOCART score improvement

from 1 month to 24 months of follow-up, i.e. from 61.8 to 84.4. Because the liquid solution allowed perfect defect filling regardless of geometry, shape, or depth, the achieved values for defect repair and defect filling rates did not change significantly due to the nature of the GelrinC procedure, resulting in an implant that was optimally integrated with the cartilage and bone tissue surrounding. From 6 to 24 months, there was a gradual increase in signal intensity and an almost full normalization of the signal intensity of the repaired tissue. This result corresponded to a tissue maturation process that was morphologically very good [26].

Global T2 index values range between 0.8 and 1.2 for normal healthy cartilage. In this study, the global T2 index value demonstrated hyaline cartilage-like collagen organization, which progress at 12, 18, and 24 months after surgery, as there were improvements in T2 index values. At 24 months, the mean value of the T2 index was 1.2-1.3 in 81 % of patients, this value was similar to that of healthy native hyaline cartilage. The achieved percentage indicates a high cure rate in patients. However, in this study, the authors did not report the side effects of GelrinC implants. Moreover, there was limited clinical evaluation, and lack of histological control data, which are the limitations of this study [26].

Wolf *et al.* used ChonDux to treat 18 individuals with articular cartilage defects of the knee. ChonDux is a hydrogel scaffold that creates a chondrogenic milieu for autologous bone marrow cells that are released by microfractures. It connects the tissue surface to a Polyethylene Glycol (PEG) hydrogel that is polymerized by long-wave ultraviolet radiation *in situ* by utilizing a chondroitin-sulfate adhesive. This scaffold fills and takes the form of irregular tissue defects, which improves integration compared to solid scaffolds that have been used previously. There was no significant variation in defect filling between any of the time points after ChonDux treatment, demonstrating that ChonDux had a steady repair potential. ChonDux maintained continuous defect filling with final defect filling reaching 94.2%$\pm$16.3 %. Between 1 and 6 weeks, the VAS pain scores were reduced, while the IKDC knee function scores increased by approximately 30.1. A total of 14 (77.8 %) patients reported side effects. Of the various side effects reports, the majority were categorized as mild or moderate, only one patient was classified as severe. Five patients had cartilage delamination, so cartilage healing occurred only in 72.2 % of

patients. The authors believe that this technique is a safe complement to microfracture therapy and that it promotes stable repair from full-thickness articular cartilage defects [27].

All-arthroscopic is a technique to repair knee cartilage defects through small incisions in the skin by inserting a special instrument through the small hole. In a study by Blanke *et al.*, 29 patients with full-size cartilage defects of the knee, all-arthroscopic hydrogel-based autologous chondrocyte transplantation was conducted. This method allows applications that do not require further fixation because the bioresorbable hydrogel attaches to the base of the defect right away, keeping the cells at the defect location, and the hydrogel-based procedure allows the cartilage defect to be entirely filled. The study reported that the 29 patients who received all-arthroscopic hydrogel-based autologous chondrocyte transplantation treatment showed similar improvement regardless of lesion size in practically all clinical scores. From preoperative to follow-up, the mean VAS was reduced considerably ($p<0.0001$). All clinical indicators i.e. IKDC, and Tegner scores, and Knee Injury and Osteoarthritis Outcome Score (KOOS) were statistically significant improved ($p<0.0001$) from baseline to follow-up. Only 6 patients in this study had minor effusion and the majority of patients (82.8 %) had a wide range of motion in their knee joint, one failure occurred in 1 patient (3.4 %) so revision surgery was performed [28].

Table 1. Clinical applications of tissue engineering in treating knee defects [17-28]

Scaffold and Cell	Number of patients with diagnoses	Follow-up Period	Clinical evaluation	Conclusion	Reference
Biomimetic osteochondral : collagen-hydroxyapatite 3 layers	27 patients with OCD of the knee of the femoral condyle	2 years	Tegner, IKDC, ICRS, MRI (MOCART)	The scaffold requires only a 1-step surgical approach and is minimally invasive cell-free. Large lesions may benefit from this implant.	[17]
Osteochondral biomimetic scaffold: biomimetic three-phase hydroxyapatite collagen nanostructure	79 patients with trochlear chondral lesions or OCD	2 years	Tegner, IKDC, MRI (MOCART)	This one-step biomimetic technique, which was created to stimulate osteochondral tissue regeneration, is successful in treating articular surface injury in the knee, resulting in considerable clinical improvement.	[18]
ACI: ACI-C, ACI-P	88 patients with knee cartilage defects	4-7 years	Lysholm , ICRS, OsScore	ACI-C produces significantly higher-quality repair tissue than ACI-P.	[19]
Biphasic osteochondral construction (DL-poly-lactide-co-glycolide)	10 patients with symptomatic osteochondral lesions of the femoral condyles	2 years	VAS, KOOS, ICRS, MRI (MOCART)	Built-in construction without additional fixation. 1-stage implantation with successful regeneration of hyaline cartilage	[20]
Arthroscopic MFX coated with collagen membranes immersed in autologous	9 patients with focal condyle lesions of the articular cartilage of the knee	1 years	Lysholm, VAS, IKDC, ICRS, MRI	The C-CMBMC technique is safe and effective in reducing pain and improving function in the short term.	[21]

BMC of the iliac crest					
Tissue-engineered cartilage-like tissue implantation	72 patients with full-thickness knee cartilage defects	5 years	Lysholm , ICRS II, MRI (MOCART)	At a median of 8.0 years after surgery, tissue-enriched cartilage-like tissue implantation for knee cartilage defects is safe and successful.	[22]
Arthroscopic MSCs with fibrin glue	24 patients with cartilage lesions of the knee, OA	2 years	Tegner, IKDC, MOAKS, MRI (MOCART)	Implanting MSCs with fibrin glue appeared to be beneficial in healing cartilage defects in knee OA patients.	[23]
Osteochondral autograft transplant (OAT)	112 patients with FCNO lesions of the knee	2 years	VAS, WOMAC Index	Middle-aged patients who are resistant to conservative treatment may benefit from short-term OAT	[24]
Costal chondrocyte–derived pellet-type (CCP) ACI	7 patients with full-thickness cartilage lesions	5 years	Lysholm, Tegner, IKDC, MRI (MOCART)	CCP-ACI is a safe and effective treatment for articular cartilage defects in terms of symptomatic improvement and knee function and structural regeneration.	[25]
GelrinC, biosynthetic hydrogel implant, biodegradable	21 patients with full thickness cartilage defects	2 years	IKDC, KOOS, MRI (MOCART)	Significant improvement during follow-up indicates cartilage tissue repair	[26]
ChonDux hydrogel scaffold	18 patients with knee articular cartilage defects	2 years	VAS, IKDC, MRI (relaksasi T2)	ChonDux treatment is a safe adjunct to micro-fracture therapy for full-thickness articular cartilage defects that promotes stable repair.	[27]

All arthroscopic hydrogel-based autologous chondrocyte transplantation	29 patients with focal full-size cartilage lesions of the knee	2 years	Tegner, VAS, IKDC, KOOS, MCID, MRI (MOCART)	Even for big diameter lesions, this minimally invasive method is a promising surgical strategy for cartilage regeneration..	[28]

Abbreviations: OCD = Osteochondritis Dissecans; ACI = Autologous Chondrocyte Implantation; ACI-C = Autologous Chondrocyte Implantation + Chondrogide; ACI-P = Autologous Chondrocyte Implantation + Periosteum; MFX = Micro-fractures; BMC = Bone Marrow Concentrate; MSCs = Mesenchymal Stem Cells; OA = Osteoarthritis; FCNO = Focal Chronic Non-traumatic Osteochondral; Tegner = score that graded activity based on work and sports activities; VAS = Visual Analog Scale; IKDC = International Knee Documentation Committee; KOOS = Knee Injury and Osteoarthritis Outcome Score; ICRS = International Cartilage Repair Society; ICRS II = ICRS Visual Assessment Scale II; MCID = Minimal Clinically Important Difference; WOMAC = Western Ontario and McMaster Universities Osteoarthritis; MOAKS = MRI Osteoarthritis Knee Score; MRI = Magnetic Resonance Imaging; MOCART = Magnetic Resonance Observation of Cartilage Repair Tissue; Lysholm = a system for examining a patient's knee-specific symptoms; OsScore = a semiquantitative scoring system.

Comparison between various methods of TE and future directions

Tissue engineering as a cartilage repair technique focuses on total articular cartilage regeneration; this approach has the ability to develop new engineered tissue to resist immune-mediated degradation and prevent future osteoarthritis progression. Certain information regarding repaired tissue structure [17-23,25,26,28], cellular morphology [19,21], proportion of hyaline cartilage formation [19,21,26,27], complete cartilage filling [17,18,20,21,23,25,28], complete integration of the graft [17-23,28], repaired tissue surface [17-24,27,28], and graft signal intensity scores [18,21,23,25,26,28] were available, but not in all studies, as only some studies reported them. Therefore, comparing all outcomes of tissue engineering methods was not feasible, due to incomplete information provided by the studies, while they should be determined in order to establish the efficacy of tissue engineering in the treatment of knee defects.

Determining the best tissue engineering method could be based on the percentage of healing achieved by patients with regenerated cartilage tissue that was hyaline cartilage and the magnitude of side effects that occurred. Unfortunately the data obtained were incomplete, and this condition was a limitation of this study. Meanwhile, the method that showed healing results with the highest percentage

of hyaline cartilage repair that was above 50% occurred in nine studies [17-20,22,23,25,27], namely 81 % in tissue engineering using GelrinC [26], while in one study the patient's recovery rate was not known [24] . Based on the magnitude of side effects that occur, the study that showed the lowest side effects was the tissue engineering method using hydrogel-based autologous chondrocyte transplantation, which was 3.4 % [28]. In term of side effects, three studies did not assess side effects [19,23,26], while three studies reported only general side effects, where none of the patients experienced serious side effects [20,24,25].

Moreover, to confirm the clinical success of tissue engineering, long-term data was required, whereas most clinical applications of scaffolds in knee defects were performed with a short follow-up period (1-2 years) [17,18,20,21,23,24,26,27]. So, it is premature to conclude that the scaffold is effective in treating knee disorder in the long term and does not require reoperation. A five-year follow-up period was applied in a clinical trial, and it was found that the incidence of partial graft detachment, graft hypertrophy, graft hardening, and graft failure was found, although there was no infection after implantation [22]. This is one of the reasons why long-term and medium-term follow-up is critical. Our opinion is supported by a review by Owida [10], who stated that short-term improvements in radiologic evaluation and clinical symptoms were less important compared to long-term recovery.

The use of a small sample [20,21,23,25,28] and the lack of a control group [17,18,21,22,24,26,28] were also limitations in most studies.

Despite the fact that many of the same procedures are utilized to treat cartilage lesions in adolescents and adults, the outcomes may vary. Older patients (>40 years) potentially have a worse outcome due to reduced regenerative capacity than younger patients (<40 years) [16]. However, in the studies that were carried out, there was no analysis of the relationship between age and the level of repair of the knee cartilage tissue.

A tissue engineering scaffold should also have structural and mechanical properties that are appropriate for the anatomic region in which it will be implanted, as well as be robust enough to allow surgical manipulation during implantation. The macrostructural properties should refer to the 3D structure that resembles the natural Extracellular Matrix (ECM) and is suitable for the phenotype of the cells to survive;

further, the microstructural properties of the scaffold need to have suitable porosity, interconnectivity, pore size, and pore shape. In addition, mechanical qualities include suitable stiffness and mechanical strength [9].

Cell type and source and the use of suitable stimuli to design a new tissue that is identical to natural articular cartilage are important to give a long-term solution for knee defects healing. Although biomimicry is the goal of tissue engineering, the technique should also seek to develop new tissues that are resistant to joint inflammation, easily integrate into surrounding native tissues, and ensure excellent results independent of biologic variability or patient age [16].

Chronic joint inflammation can damage tissue-engineered implants, which make them difficult to integrate and perform. Although articular cartilage is immune privilege, the degree of immunological privilege granted to an implant relies on its location within the knee joint and closeness to the synovium [29].

ACI, Matrix-induced Autologous Chondrocyte Implantation (MACI), and OAT are examples of current cell-based cartilage regeneration procedures that allow implants to be molded to recipient defects. Despite the fact that the study's findings indicated an increase in knee function and a high degree of satisfaction, unfortunately this technique requires 2 stages of surgery, namely chondrocyte removal or bone marrow concentrate removal or tissue removal (operation 1) and implantation into the chondral defect (operation 2) [19-25]. This opinion is supported by Jiang *et al.* review [14]. Moreover, they reported that a systematic review that enrolled a large number of knee cartilage defect patients showed that MACI was no better than ACI or OAT [14].

Various synthetic and natural materials, such as a decellularized cartilage-derived matrix, injectable hydrogels that can fill irregular defects, and a porous polymer structure that mimics the entire osteochondral anatomy, have been employed as scaffolds for manufactured knee articular cartilage. This option of course minimizes the use of invasive implantation methods and reduces the stages of surgery [17,18,26,28].

Scaffold-free engineered cartilage provides promising potential. Scaffold-free engineered cartilage has acquired functional qualities comparable to native tissue by using biochemical and biomechanical stimulation. Because articular cartilage is avascular, it is difficult to integrate the implant with the existing

native tissue. As implant integration, surgical technique, and rehabilitation all contribute to cartilage regeneration efficacy, researchers should focus on developing optimal protocols to address these issues as well as implant development [16].

CONCLUSION

Tissue engineering has made significant progress in the treatment of knee defects. Based on the results of this study, the method that showed the best healing results was tissue engineering using GelrinC which was 81 %, and the method that had the lowest side effects was tissue engineering using hydrogel-based autologous chondrocyte transplantation, which was 3.4 %. Research and development of tissue engineering is still needed to obtain high healing results without postoperative side effects.

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