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REVIEW

Articular cartilage repair and the evolving role of regenerative medicine

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Abstract: Among the growing applications of regenerative medicine, clinical articular cartilage repair has now been used for 2 decades and forms a successful example of translational medicine. Cartilage is characterized by a limited intrinsic repair capacity following injury. Articular cartilage defects cause symptoms, are not spontaneously repaired, and are generally believed to result in early osteoarthritis. Marrow stimulation techniques, osteochondral transplantation, and cell-based therapies, such as autologous chondrocyte implantation (ACI) and use of mesenchymal stem cells (MSCs), are used for tissue regeneration, symptom relief, and prevention of further joint degeneration. The exact incidence of cartilage defects and the natural outcome of joints with these lesions are unclear. Currently available cartilage repair techniques are designed for defect treatment in otherwise healthy joints and limbs, mostly in young adults. The natural history studies presented in this review estimated that the prevalence of cartilage lesions in this patient group ranges from 5% to 11%. The background and results from currently available randomized clinical trials of the three mostly used cartilage repair techniques are outlined in this review. Osteochondral transplantation, marrow stimulation, and ACI show improvement of symptoms with an advantage for cell-based techniques, but only a suggestion that risk for joint degeneration can be reduced. MSCs, characterized by their good proliferative capacity and the potential to differentiate into different mesenchymal lineages, form an attractive alternative cell source for cartilage regeneration. Moreover, MSCs provide a regenerative microenvironment by the secretion of bioactive factors. This trophic activity is believed to limit damage and stimulate intrinsic regenerative responses. Finally, important clinical issues are discussed, including techniques to study the role of implanted cells in tissue regeneration using cell labeling and cell tracking, the improvement of cartilage integration, the use of delayed gadolinium-enhanced magnetic resonance imaging of cartilage for early judgment of joint degeneration/regeneration, and the influence of regulatory rules for therapeutic application development.

Keywords: articular cartilage, repair, imaging, techniques

Introduction

Cartilage is characterized by a limited intrinsic repair capacity following injury. Articular cartilage lesions are frequently associated with symptoms such as pain, effusion, locking phenomena, and disturbed function. Moreover, these lesions are generally believed to progress to early osteoarthritis (OA).[1–3] Regenerative medicine, including in situ induction of cartilage tissue, use of tissue-engineered cartilage constructs, or cell-based therapies (autologous chondrocytes or mesenchymal stem cells [MSCs]) is used for tissue regeneration, symptom relief, and prevention of further degeneration.

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Mankin, in his editorial accompanying Brittberg's pioneering paper in the *New England Journal of Medicine* on autologous chondrocyte implantation (ACI) in 1994,[4] discussed the difficulties of treating cartilage injuries and stated: the tissue is difficult to work with, injuries to joint surface – whether traumatic or degenerative – are unforgiving, and the progression to OA is sometimes so slow that we delude ourselves into thinking we are doing better than we are[5] clinical and basic scientists have made much progress since 1994 in understanding cartilage disease and degeneration and also have made progress in biological repair of it. However, Mankin's remarks illustrate that the translation from basic knowledge and experimental treatments toward *successful* and *durable* repair of cartilage defects and osteoarthritic joints is difficult. The treatment goals of cartilage pathology are symptom relief, improvement of joint congruence by restoring the joint surface with the best possible tissue, and prevention of further joint degeneration.

Articular cartilage is a highly organized avascular tissue composed of chondrocytes embedded within an extracellular matrix of collagens, proteoglycans, and noncollagenous proteins. It makes painless, low friction movement of synovial joints possible. Hyaline cartilage covers the subchondral bone and forms the articulating surface of synovial joints. It functions as a mechanical shock absorber and distributes the applied load over the subchondral bone. The regeneration capacity of articular cartilage following injury is considered to be limited. Partial-thickness articular cartilage defects, limited to the cartilage itself, are not repaired, and full-thickness defects are repaired with fibrocartilage,[6] which has inferior biological and biomechanical properties compared with hyaline cartilage.[7]

In this review, we will give a description of the epidemiology and natural history of cartilage lesions and provide an overview of current regenerative cartilage repair techniques, review outcomes from randomized clinical trials (RCTs), and give insight on new developments with use of MSCs and tissue-derived progenitor cells.

Epidemiology and natural history of cartilage injury

The exact incidence of symptomatic chondral lesions and the natural outcome of joints with osteochondral lesions are not well defined. A prospective study of 1,000 consecutive knee arthroscopies revealed International Cartilage Repair Society (ICRS) grades III and IV chondral lesions,[8] with an area of at least 1 cm^2, in patients younger than 40, 45, and 50 years of age (5.3%, 6.1%, and 7.1%, respectively).[9] The mean osteochondral defect area was 2.1 cm^2. Another prospective study of 993 knee arthroscopies[10] in patients with a median age of 35 years shows that 11% had full-thickness articular cartilage defect (ICRS grades III and IV), and 6% had a lesion size of more than 2 cm^2. Prospective arthroscopic evaluation of traumatic knee hemarthrosis patients showed 8%–20% osteochondral lesions, frequently associated with injury to the anterior cruciate ligament (ACL).[11–13] Curl et al[14] retrospectively reviewed 31,516 knee arthroscopies of patients in all age groups and reported chondral lesions in 19,827 (63%) patients, with a mean of 2.7 lesions per knee. The incidence of grade III lesions was 41% and grade IV lesions 19%. In the younger population (age <40 years), however, the incidence of unipolar grade IV lesions of the femoral condyle was only 5%. Thus, the prevalence of isolated osteochondral defects ranges from 5% to 11% in the young patients and up to 63% in the patients overall.

It is unclear which chondral lesions give symptoms. Many of the detected lesions are asymptomatic and, therefore, the exact incidence in the general population is unknown. It is likely that symptoms and joint degeneration are dependent on lesion size, location, and patient characteristics.

Shelbourne et al[15] reported a series of 125 Outerbridge grades III and IV[16] cartilage defects (mean size 1.7 cm^2, 60 medial, 65 lateral compartment, and intact menisci) discovered during 2,770 ACL reconstructive procedures. These authors showed, at a mean follow-up of 8.7 years, very little difference in clinical outcome following ACL repair between patients with a chondral defect and those without a defect. There was no difference between groups with regard to radiological degenerative changes.

However, it may take up to more than 20 years before clinical and radiological degenerative changes come forward. Linden[17] showed in a retrospective radiological study on osteochondritis dissecans (OCD) of the knee in adult patients, with a 32.5 ± 7.5 year follow-up, mild radiological deterioration in 14/44 joints and severe changes in 29/44 joints.

In a recent natural history study in 2010, Widuchowski et al[18] retrospectively analyzed 4,121 consecutive knee arthroscopies. In the patient group younger than 35 years, there were 37 single-isolated Outerbridge grades III and IV lesions within the weight-bearing areas of the femorotibial compartments and the patella. At a mean follow-up of 15.3 years, the authors found no difference in OA severity between the injured and the uninjured knees, indicating that severe isolated chondral defects may have limited influence on the development of knee OA.

Gelber et al[19] followed 1,321 former medical students with joint injuries, with median follow-up of 36 years, and found that 13.9% of students with knee injuries progressed to fully developed knee OA by the age of 65 years compared with 6% in controls without joint injury. In this frequently cited article, however, from the 111 isolated knee injuries, just 8 sustained an isolated cartilage injury. More severe injuries including tibial plateau fractures, knee dislocation, and open fractures are considered to have a higher risk for OA development.

In conclusion, most authors assume that cartilage lesions, frequently associated with other articular injuries, progress to joint degeneration. However, it is difficult to exactly predict which lesions will benefit from cartilage repair. Whether a cartilage lesion causes progression toward OA may depend on lesion size, location, preinjury joint degeneration, limb alignment, and other patient characteristics.

The available articular cartilage repair techniques are not designed to treat degenerative joint disease, eg, OA. However, several authors have used the described techniques or combinations of it to treat degenerative joint disease.[20,21] For example, they used microfracturing[20] and/or meniscus transplantation or joint realignment procedures for knee OA cases.[21] To date, there are no randomized clinical studies reporting the outcome of treatment of these patient groups.

OA affected nearly 27 million or 12.1% of the adult population in the United States in 2008.[22] OA is the fifth leading cause of disability in older Americans after cardiovascular, cerebrovascular, and pulmonary diseases.[23] It is estimated that the number of adults in the United States with arthritis disease will reach up to 67 million, or 25% of the population, by 2030.[24] Successful repair techniques for isolated cartilage defects in otherwise healthy joints may, in the future, be translated to treatment of more extensive joint degeneration such as OA. Biological repair and possible disease modification, with the use of regenerative medicine techniques, may thereby decrease the expected medical and economic burdens.

Current clinical methods of repair

The primary goal in articular cartilage repair procedures should be defect filling and restoration of the articular surface with the best possible repair tissue. Long-lasting biomechanical properties resembling that of hyaline cartilage and a full integration with the surrounding articular cartilage should result in pain-free movement and prevent early joint degeneration. Surgical treatment options for cartilage repair include symptomatic treatments like debridement and lavage, osteochondral autograft transplantation (OAT), marrow stimulation techniques (Pridie drilling or microfracture), ACI, and tissue engineering techniques using cells and biomaterials to replace damaged or lost cartilage and bone.

Focusing on articular cartilage repair, we can distinguish three main techniques for biological repair of cartilage defects: osteochondral transplantation (OAT or mosaicplasty), subchondral marrow stimulation (Pridie drilling or microfracture), and ACI.

In OAT or mosaicplasty, introduced in the 1990s, autologous osteochondral biopsy plugs are harvested from relatively nonweight-bearing areas of the joint and subsequently implanted in a mosaic-like pattern in debrided cartilage defects.[25,26] OAT or mosaicplasty, popularized by Hangody et al[25] and Bobic,[26] is recommended for defects limited to between 1 and 4 cm^2.[27] For these small- to medium-sized defects, good results have been reported in terms of function scores and histology for follow-up up to 7 years after treatment.[25,27] Drawbacks of this technique are limited availability of donor tissue and donor site morbidity.[25,27,28] In this review, we will focus on the two techniques that can be characterized as regenerative medicine: the microfracture technique and ACI with or without matrix augmentation.

Microfracture

Already in the 1950s, it was hypothesized that accessing the bone marrow could be helpful in the repair of cartilage defects. The general hypothesis behind all marrow stimulation techniques is that MSCs present in the bone marrow are responsible for the formation of fibrocartilaginous tissue that fills the initial defect.[6,29] Marrow stimulation in the microfracture technique is achieved by cortical penetration with an awl; in Pridie drilling, a drill or Kirchner-wire is used. The microfracture technique was introduced in the 1980s and is considered as an evolved form of Pridie drilling, and generally accepted to result in clinically more favorable outcomes, which is attributed to the absence of thermal damage in the microfracture technique, although the two techniques have never been compared directly. After debridement of the defect, conical holes of 0.5–1 mm in diameter and 4 mm deep are punched all over the defect at a distance of 3–4 mm apart with specialized tapered awls. Consequently, a blood clot fills the defect followed by ingrowth of bone marrow cells.[30–34]

Using the equine medial femorotibial joint as a model for the medial femoral condyle of the human knee, many aspects of the microfracture technique have been studied.[35–40] A finding that was subsequently translated into clinical practice was the importance of the removal of the calcified cartilage layer prior to creation of the holes.[36,39] Insufficient removal can lead to dislocation of the clot, where damaging the subchondral plate can result in overgrowth of subchondral bone, causing decrease of repair tissue volume and compromised mechanical properties.[36,39,41,42]

For optimal results, patients should preferably be younger than 45 years of age, have a body mass index less than 30 and experience symptoms (activity related pain, swelling, locking, and catching) for less than 1 year. The defect should be isolated from other lesions and should be smaller than 4 cm^2. An intact rim of cartilage should surround the defect to ensure that the bone marrow clot stays in place.[33] Contraindications are degenerative joint changes, axial malalignment $>5°$ for femoral condyle defects, tumors, infections, meniscus pathology that requires treatment, and high-grade ligament instabilities.[33,36] Microfracture is often coupled to a specific rehabilitation program.[43] Initially, weight-bearing is avoided, followed by controlled partial weight-bearing, to provide nutrients and to provide mechanical stimuli.[33,34,43–46] Return to full premorbid activities is generally achieved at 15–18 months after surgery.[43] Improvement in terms of pain and function is widely reported up to 24 months after surgery; however, the long-term durability is debated; return of complaints is generally expected. Upon histological analysis of biopsies taken at 2 years after treatment, 69% of treated lesions were found to consist of mainly fibrocartilaginous tissue, whereas 11% predominantly contained hyaline cartilage.[47] The fibrocartilaginous repair tissue contains more type I collagen and less proteoglycan compared with native articular cartilage, indicating that the biochemical and biomechanical properties are not equal to those of the native articular cartilage.[35,37–39,48]

Many augmentation strategies are currently being investigated in order to improve the long-term outcome of the microfracture technique, eg, autologous matrix-induced chondrogenesis (AMIC) involving type I/III collagen scaffolds (Geistlich Pharma AG, Wolhusen, Switzerland) or chitosan-glycerol phosphate-based BST-CarGel® scaffold (Biosyntech Inc, Laval, Canada). Good results were reported for the AMIC type I/III collagen scaffold technique.[49] Improvement of repair tissue quality and ICRS II scores compared with conventional microfracture has been found for chitosan-glycerol phosphate-based BST-CarGel, which is currently being evaluated in a multicenter clinical trial.[50–53] Other augmentation strategies consist of hyaluronic acid injections or biomaterials with incorporated growth factors.[32,54–57] Most of these strategies are currently in preclinical stages.[56]

Autologous chondrocyte implantation

The ACI technique for clinical treatment of human cartilage defects was first reported in 1994.[4] In summary, a cartilage biopsy is taken arthroscopically from a nonweight-bearing area of the joint. Cartilage biopsies are enzymatically digested to isolate chondrocytes. The chondrocytes are expanded in monolayer culture. In a second open procedure that can take place 6 weeks up to 18 months after the biopsy, a periosteal flap, harvested from the tibia, is placed over the cartilage defect, fixed with sutures, and sealed with fibrin glue after which a solution of expanded chondrocytes is injected underneath the flap.[4,58] This first-generation ACI procedure is also known as ACI-P, based on the use of the periosteal flap. Leakage of cells, uneven distribution of chondrocytes, and hypertrophy of the periosteal flap were reported.[58,59] In the second-generation ACI procedure, a collagen membrane is used to replace the periosteal flap, which is also known as ACI-C.[58] Several tissue engineering-based approaches are classified as the third generation of ACI, which means that cells are cultured on a biodegradable membrane or scaffold prior to placement into the defect, eg, matrix-induced ACI (MACI). In this MACI technique, chondrocytes are precultured on a porcine type I/III collagen membrane. Another approach is the use of characterized chondrocyte implantation (CCI) marketed as ChondroCelect® (TiGenix NV, Leuven, Belgium), in which autologous chondrocytes are characterized based on specific marker proteins, and expansion is standardized.[60,61] With these emerging tissue engineering strategies, the possibility to perform ACI procedures arthroscopically gains more interest.[60,62,63] The presence of cartilage damage at the opposing surface of the joint is a contraindication for ACI.[58] Malalignment and ligamentous instability should be corrected prior to treatment.[58,64] For optimal results, prevalence of symptoms should be less than 2 years, and the cartilage defect should be an isolated focal lesion.[58] As for microfracture, an intact rim of cartilage should surround the defect to allow suturing of the periosteal flap, membrane, or scaffold. Damaging the subchondral bone is to be avoided to prevent the formation of fibrocartilaginous tissue due to bone marrow invasion.[58] In contrast to the microfracture technique, for ACI no relationship between defect size and clinical outcome was found, which implies that ACI can be applied for cartilage defects of all sizes.[65] ACI treatment of cartilage defects is, as for microfracture, followed by a rehabilitation program involving restricted weight-bearing and use of continuous passive motion. Return to daily activities and light sports is generally achieved at 4–6 months after treatment.[58,66] Good to excellent outcomes have been reported for long-term (up to 7 years) follow-up of femoral condyle lesions treated with ACI, with indications of clinical outcome improvement over the years.[66] Histological biopsy studies[67] have shown that ACI repair can result in repair tissue of varying morphology, ranging from predominantly hyaline (22%) through mixed (48%) to predominantly fibrocartilage. The success rate of ACI reported in long-term durability studies varies from 69% at 10-year follow-up[68] to 84% at 7.4-year follow-up.[66] Failures are reported within the first years following treatment; good results at short-term follow-up are generally sustained at long-term follow-up.[66,68]

ACI was also found to result in improved clinical outcome when applied in patients with large cartilage defects that failed to repair in a previous treatment.[69] However, this finding is debated; poor clinical outcome has been reported for patients undergoing ACI or MACI after failing mosaicplasty or ACI.[70] A limitation in ACI and its derivatives is the fact that chondrocytes tend to dedifferentiate during monolayer expansion, which decreases their extracellular cartilage matrix formation potential.[71] For ACI, a widely used animal model is lacking due to reported variations in expansion and other characteristics of autologous chondrocytes over different species.[72]

Among the various types of cartilage, of interest are two distinct different cartilage types in the growing skeleton: (1) growth plate cartilage and (2) hyaline or articular cartilage. In growth plate cartilage, chondrocytes proliferate, become hypertrophic, and terminally differentiate. This process of maturation leads to cell death, followed by calcification of the tissue, and chondrocytes are replaced by osteoblasts. In the growing joint hyaline cartilage, chondrocytes proliferate and form the extracellular matrix, resulting in adult hyaline cartilage, with chondrocytes lying in low densities in a tight extracellular matrix. For ACI regenerative cartilage medicine, one of the challenges is to culture-expand cells to sufficient numbers for tissue regeneration while preventing hypertrophy, terminal differentiation, and calcification of the repair tissue.[73]

Randomized clinical trials

Many case studies and clinical trials on the above-described techniques have been published during the last 2 decades. In addition, several (systematic) reviews on ACI or cartilage repair techniques in general have been published during recent years[2,32,36,48,64,74–81] expressing the growing interest of the community in the possibilities of these techniques. For this review, we decided to highlight the RCTs. The microfracture technique, popularized by Steadman,[36] is often used as a first treatment for cartilage defects and has become the control treatment in several prospective studies evaluating other more extensive surgical interventions such as ACI. We aim to provide an overview of RCTs involving ACI or its derivatives, microfracture, or both. The study and patient characteristics of all available RCTs are shown in Table 1. The outcome parameters and RCT quality scores are summarized in Table 2. The quality of presented RCTs comparing the different cartilage repair techniques was assessed according to Jadad et al.[82]

In 2003 Horas et al[83] conducted a prospective RCT in which femoral condyle lesions of 40 patients were treated either with ACI-P or OAT. For both treatments, improvement of Lysholm Knee Scoring Scale (LKSS) scores compared with preoperative levels was reported; however, the increase was significantly slower for ACI-P-treated patients compared with OAT-treated patients at follow-up after 6, 12, and 24 months.[83]

In 2003 Bentley et al[84] reported their findings of a prospective RCT in which 100 patients with symptomatic lesions of the articular cartilage of the knee were treated either with ACI-P/C or mosaicplasty. At 1 year after treatment, 82% of the ACI-P/C-treated group showed good or excellent results in ICRS grade arthroscopic results against 34% of the mosaicplasty-treated group. Upon functional assessment (Cincinnati and Stanmore scores), 88% of the ACI-P/C-treated group had good or excellent results, whereas in the mosaicplasty, 69% was reported. It has to be noted that in this study patients with cartilage lesions over 4 cm^2 in size were treated with mosaicplasty. Also, the rehabilitation program was similar for both treatment groups, whereas different programs are recommended for ACI and mosaicplasty.[27,84]

Bartlett et al[70] reported their results of a prospective RCT study of ACI-C vs MACI in 2005. Ninety-one patients were randomized to one of the treatments. No significant differences were observed at 1 year after treatment in terms of ICRS scores, histological examination, and functional Cincinnati knee scores. Bartlett et al[70] concluded that although no significant differences were found between the two treatments, MACI is technically more attractive due to factors like quicker surgery and the possibility not to use sutures; however, more long-term studies are required.

ACI-P and mosaicplasty were compared in a multicenter RCT by Dozin et al in 2005.[85] Forty-seven patients were treated with arthroscopic debridement and subsequently randomized to one of the treatments. Debridement alone resulted in improvement to such an extent that 14 patients (31.8%) were clinically asymptomatic and were not subjected

Table 1 Overview of RCTs on cartilage defect repair techniques

Author	Treatments (n)	Patient age (y)	Defect size (cm^2)	Longest follow-up, mo	Outcome measures
Horas et al[83]	ACI-P (20) OAT (20)	31.4 (18–42) 35.4 (21–44)	3.9 (3.2–5.6)	24	LKSS Tegner SEM Histology
Bentley et al[84]	ACI-P and ACI-C (58) MP (42)	30.9 (16–49) 31.6 (20–48)	4.66 (1–12) 4.66 (1–12)	12	Cincinnati Stanmore ICRS Histology
Bartlett et al[70]	ACI-C (44) MACI (47)	33.7 (15–49) 33.4 (17–47)	6 (1.5–16) 6.1 (1–22)	12	Cincinnati Stanmore VAS Histology
Dozin et al[85]	ACI-P (22) OAT (22)	29.6 (±7.3) 27.9 (±8.1)	1.97 (±0.43) 1.9 (±0.45)	36	LKSS IKDC
Gudas et al[86]	OAT (28) MF (29)	24.6 (±6.54) 24.3 (±6.8)	2.8 (±0.65) 2.77 (±0.68)	36	ICRS HSS MRI Histology
Gooding et al[87]	ACI-P (33) ACI-C (35)	30.5 (15–52) 30.5 (16–49)	4.54 (1–12)	24	Cincinnati ICRS Histology
Knutsen et al[47,88]	ACI-P (40) MF (40)	33.3 31.1	5.1 4.5	60	LKSS Kellgren and Lawrence ICRS Tegner SF-36 Histology
Saris et al[61,89]	CCI (57) MF (61)	33.9 (±8.5) 33.9 (±8.6)	2.5 (1–5)	36	KOOS MRI

Notes: Characteristics on treatment groups, number of patients per treatment, defect sizes, duration of after treatment follow-up, and the applied outcome measures are presented.

Abbreviations: RCTs, randomized clinical trials; ACI-P, first generation autologous chondrocyte implantation (with periosteal coverage); OAT, osteochondral autograft transplantation; LKSS, Lysholm Knee Scoring Scale; SEM, scanning electron microscopy; ACI-C, second generation autologous chondrocyte implantation (with collagen coverage); MP, mosaicplasty; ICRS, International Cartilage Repair Society Score; MACI, matrix-induced ACI; VAS, visual analog scale for pain; IKDC, International Knee Documentation Committee Scale; MF, microfracture; HSS, hospital for special surgery knee score questionnaire; MRI, magnetic resonance imaging; CCI, characterized chondrocyte implantation; KOOS, Knee injury and Osteoarthritis Outcome Score.

to further treatment. Eventually, 52.3% of the originally included patients were evaluated. For 88% of the patients subjected to mosaicplasty and 68% of the ACI-P-treated patients complete clinical recovery was reported. The 2 methods were found to be clinically equivalent.

OAT and microfracture were compared in a prospective RCT in competitive or well-trained athletes by Gudas et al in 2006.[86] Fifty-seven patients with full-thickness cartilage lesions or single OCD were randomized to either OAT or microfracture. The recommended continuous passive motion rehabilitation program was not applied. At 6, 12, 24, and 36 months after treatment, patients were evaluated. 93% of the OAT and 52% of the microfracture-treated group returned to presymptomatic sports level at 4–8 months after treatment. In terms of hospital for special surgery (HSS) and ICRS scores, magnetic resonance imaging (MRI), and histology and clinical assessments, significantly better results were reported for OAT compared with microfracture-treated athletes.[86]

In 2006, Gooding et al compared ACI-P with ACI-C in a prospective RCT including 68 patients.[87] In 36.4% of the ACI-P vs none in the ACI-C-treated patients graft hypertrophy occurred, and shaving was required at 1 year after treatment. No significant differences between the 2 treatments were found in terms of Cincinnati, ICRS, and histology at 2 years after treatment.[87]

Knutsen et al[47,88] reported their findings at 5 years in 2007 of a multicenter RCT comparing ACI-P with microfracture in 80 patients with single-cartilage defects of the femoral

Table 2 Quality assessment and summary of the main conclusion of the RCTs

Author	Treatments (n)	RCT quality score	Results
Horas et al[83]	ACI-P (20) OAT (20)	3	Meyer and Tegner: equal results LKSS improvement both treatments, slower for ACI-P
Bentley et al[84]	ACI-P and ACI-C (58) MP (42)	2	ICRS score good/excellent: ACI-P 82%, MP 34% Cincinnatti/Stanmore good/excellent: ACI-P 88%, MP 69% Clinical outcome: ACI-P > MP
Bartlett et al[70]	ACI-C (44) MACI (47)	1	Cincinnati/Stanmore, VAS, histology: equal results
Dozin et al[85]	ACI-P (22) OAT (22)	3	31.8% no further treatment needed following debridement Complete clinical recovery: ACI-P 68%, OAT 88% Clinical outcome: equal
Gudas et al[86]	OAT (28) MF (29)	2	ICRS and HSS good/excellent: OAT 96%, MF 52% Clinical outcome: OAT > MF
Gooding et al[87]	ACI-P (33) ACI-C (35)	2	Cincinnati, ICRS, histology: equal results Graft hypertrophy: ACI-P 36.4%, ACI-C 0%
Knutsen et al[47,88]	ACI-P (40) MF (40)	3	LKSS, Kellgren and Lawrence, ICRS, Tegner, SF-36, histology: equal results 77% satisfactory results and 23% failures in both groups No relation between histology and clinical outcome
Saris et al[61,89]	CCI (57) MF (61)	3	KOOS: both significant improvement, CCI > MF

Notes: A summary of the results and conclusions are displayed per RCT. The quality of presented RCTs was assessed using the Jadad score[82] (0–5, a high score is an indication of high quality of the study). The comparison of single surgery approaches (MF, OAT) with double surgery approaches (all ACI) makes double blinding the procedure difficult for these surgical interventions; without double blinding the maximum Jadad score for RCTs is 3.

Abbreviations: RCTs, randomized clinical trials; ACI-P, first generation autologous chondrocyte implantation (with periosteal coverage); OAT, osteochondral autograft transplantation; LKSS, Lysholm Knee Scoring Scale; ACI-C, second generation autologous chondrocyte implantation (with collagen coverage); MP, mosaicplasty; ICRS, International Cartilage Repair Society Score; MACI, matrix-induced ACI; VAS, visual analog scale for pain; MF, microfracture; HSS, hospital for special surgery knee score questionnaire; CCI, characterized chondrocyte implantation; KOOS, Knee injury and Osteoarthritis Outcome Score.

condyle of the knee. After 5 years, 23% failures were reported in both groups, defined as reoperation required due to symptoms as a result of lack of healing after the initial treatment. Shaving or trimming was necessary in 25% of the ACI-P and 10% of the microfracture-treated patients.[47] This was not considered failure. No significant differences were found between both treatments in terms of clinical and radiographical outcome. Interestingly, no relation between histological findings and clinical outcome was observed.[88]

The results of a multicenter RCT comparing CCI (ChondroCelect) with microfracture at 36 months after treatment were reported by Saris et al[61,89] in 2008 and 2009. One hundred and eighteen patients with symptomatic lesions of the femoral condyle of the knee were randomized to one of the treatments. Based on findings in characterization of the autologous chondrocytes, 6 patients were not subjected to CCI treatment. It is not known whether these chondrocytes classified as not usable actually can result in compromised repair tissue. It was found that the longer the duration of the symptoms, the higher the improvement was of the Knee injury and Osteoarthritis Outcome Score (KOOS) of CCI-treated patients compared with microfracture-treated patients. The clinical outcome in terms of KOOS at 36 months after treatment showed significantly better results for CCI compared with microfracture-treated patients.[89]

Evaluating the currently available RCTs, within the two main objectives of articular cartilage repair, symptom relief and prevention of joint degeneration, we can conclude that all therapies show initial improvement measured with functional outcome scores. The comparison of single surgery approaches (microfracture, OAT) with double surgery approaches (all ACI) makes double blinding the procedure difficult for these RCTs. Therefore, none of the RCTs reached the maximum Jadad RCT quality score of 5 points; 4 of 8 RCTs scored the maximum of 3 points for unblinded RCTs and could be regarded as good quality RCTs. Considering prevention of early OA development, histological examinations have shown variable results, with a general suggestion of better tissue quality following ACI compared with microfracture and OAT. Arguments to assume that microfracture and OAT may result in worse long-term outcome and earlier OA development are fibrocartilage formation, donor-site morbidity, and the persistence of gaps between osteochondral plugs and surrounding cartilage. However, in general ACI is a two-stage procedure (harvesting of cartilage and a second open/arthroscopic implantation procedure) that may also lead to complications. For example, an increased inflammatory response and negative influences on joint propriocepsis following two procedures performed shortly after each other may also increase the risk for early OA development. The above-mentioned natural outcome studies have shown that it takes a long time for untreated cartilage defects to lead to detectable OA. Therefore, it may even take longer to judge whether extensive cell-based interventions protect joints from degenerating. Moreover, marrow stimulation shows good improvement in short- to mid-term follow-up. This may already be sufficient to protect joints from degeneration. Use of MRI techniques, such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), or sensitive biomarkers for OA development are examples by which we can improve early judgment of cartilage repair tissue.

MSCs for cartilage repair

All the above-mentioned techniques make use of chondrocytes for articular cartilage repair with the exception of microfracture, whereas MSCs are accredited for the formation of repair tissue. For the treatment of cartilage defects, cells are needed in substantial amounts to fill the gap and to produce extracellular matrix of sufficient strength in a relatively short time compared with cartilage development in a growing joint. This requires in vitro cell expansion of harvested and enzymatically liberated chondrocytes with the risk of dedifferentiation and loss of redifferentiation capacity after expansion.

MSCs can be an attractive alternative cell source. MSCs have a good proliferative capacity in culture and have the potential to differentiate into different mesenchymal lineages, such as bone, cartilage, tendon, muscle, and fat.[90] Chondrogenic differentiation is achieved when the cells, after expansion, are allowed to form three-dimensional aggregates in a chemically defined medium-containing transforming growth factor-β (TGF-β) and dexamethasone.[91]

These multipotent progenitor cells can be derived from several tissues, including bone marrow,[92] adipose tissue,[93] joint-related tissues like synovial membrane,[94] and infrapatellar fat.[95] Furthermore, it has been shown that articular cartilage contains progenitor cells with the capacity to regenerate cartilage in vitro.[96] In another study, undifferentiated progenitor cells were isolated from 7-day-old calf articular cartilage.[97]

Subsequent to their capacity to form repair tissue, MSCs have been shown to secrete a large spectrum of bioactive molecules in culture, including TGF-β, interleukin (IL)-10, IL-6, lymphocyte inhibitor factor, cyclooxygenase (COX)-1, and COX-2.[98] These molecules are immunosuppressive; therefore, the secreted bioactive molecules are believed to provide a regenerative microenvironment for injured or ischemic adult tissues. This regenerative microenvironment referred to as trophic activity, provided by the presence of MSCs, limits the damage sustained by injury or ischemia and stimulates intrinsic regenerative responses.[99]

For similar reasons, MSCs have been shown to be a promising cell population for immunomodulatory therapy as they can modulate T-lymphocyte reaction both in vitro and in vivo.[100] Le Blanc et al[101] showed that *ex vivo*-expanded allogeneic MSCs were immunosuppressive, reverse established graft vs host disease, and prolonged graft survival in patients after bone marrow transplantation. MSC infusions have also been tested as a possible method to induce immunologic tolerance or to reduce the need for pharmacologic immunosuppression for organ transplantation.[100]

Furthermore, from cardiovascular research, we have learned that the microenvironment provided by injected MSCs, and not the initially believed transdifferentiation of MSCs into contractile cardiomyocytes, reduces the development of heart failure following myocardial infarction.[102] From this point of view, one can hypothesize that a part of the observed effects of current cartilage repair techniques depend on this trophic activity. Undifferentiated or dedifferentiated cells, actors in the observed repair with microfracture and ACI techniques, may not only inhabit the cartilage defects and produce the necessary extracellular matrix but also provide a regenerative microenvironment. This may partly explain why investigators have found no distinct relation between repair cartilage histology and functional outcome.[88] Future studies have to reveal whether we can further improve these techniques by optimizing trophic activity. Catabolic conditions in joints with cartilage defects and/or OA may be stopped or reversed by the continuous presence of MSC trophic activity.

Animal studies, using a combination of MSCs combined with different biomaterials and growth factors, have shown promising results.[103] There are few clinical case-studies reporting the results of bone marrow-derived MSCs for cartilage defect repair. The MSCs are implanted in cartilage defects, seeded in collagen, and covered with periosteum.[104] Others have injected culture-expanded MSCs percutaneously into the knee in an attempt to regenerate cartilage in OA patients.[105] Currently, it is not known whether MSC treatments can give results similar to ACI or microfracture treatment.[77] A recent observational cohort study compared bone marrow-derived MSCs with chondrocytes and found no differences in clinical outcome scores.[106] The authors concluded that bone marrow-derived MSCs were as effective as chondrocytes for articular cartilage repair, with the advantage of one fewer knee intervention and minimized donor-site morbidity.

Clinical issues and future perspectives

Which cells are responsible for repair?

The presented cartilage repair techniques are designed to replace damaged articular cartilage, by supplying or attracting cells in sufficient amounts that produce extracellular matrix and thereby fill the gap. There is evidence to support the idea that the implanted culture-expanded chondrocytes or MSCs are relevant for cell-based therapies.[107,108] In order to further optimize cell-based therapies, we need to know whether the implanted cells can be accredited for repair tissue formation by cell tracing in the joint. The fate of cells following in vivo implantation in humans and their exact role in regeneration remain unclear. It may appear that other cells are relevant for repair tissue formation, cells such as periosteal progenitor cells in ACI-P, ingrowth of subchondral marrow-cells, or synovial progenitor cells. Studies undertaken to determine the fate of implanted chondrocytes for in vivo follow-up include retroviral green fluorescence protein marking of cells,[109] PKH26 fluorescent labeling of chondrocytes,[107] and the use of "physicochemical labels" such as magnetic nanoparticles.[110,111] An important advantage of magnetic nanoparticles like "superparamagnetic iron oxide"-labeling over other labeling techniques is that it enables clinical noninvasive in vivo cell tracking using MRI, without the need for harvesting biopsies. This allows for continuous follow-up of biological repair of articular cartilage without influencing the repair tissue or jeopardizing the patient with repeated interventions.

Integrative cartilage repair

An important prerequisite for durable repair of cartilage lesions is the integration of regenerated or transplanted cartilage with the surrounding native cartilage at the recipient site. Integrative cartilage repair is probably hindered by the lack of matrix-producing cells in wound edges caused by chondrocyte death induced by wounding of cartilage. In vitro experiments have shown a rapid onset of cell death in experimentally wounded hyaline cartilage.[112,113] The acellularity is probably caused by a combination of chondrocyte loss from lesion edges, cartilage avascularity, absence of necrotic tissue removal, and the inability of chondrocytes to migrate through the tight extracellular matrix. Approaches to improve cell density at wound edges include use of cells in the interface region, enzymatic treatment, and use of immature constructs. Silverman et al[114] studied the adhesion between native cartilage discs, using fibrin glue polymer alone or mixed with fresh articular chondrocytes. Their results demonstrated that adhesion of cartilage-to-cartilage can be improved by an increased amount of chondrocytes in the interface region.

The age and cell density of regenerated tissue influence the histological integration and its biomechanical strength. An increased cell density in the interface region by using immature constructs compared with mature constructs in an integration study was shown to improve biomechanical bonding strength and histological integration.[115] In other studies highly purified collagenase treatment was used, which resulted in an increased wound edge cell density. This "vitalization" of wound edges with vital, matrix-producing cells at the cartilage-to-cartilage interface was shown to improve histological and biomechanical integrative cartilage repair.[112,116,117] Enzymatic removal of proteoglycans or glycosaminoglycan (GAG) chains from the cartilage lesion edges by using chondroitinase ABC, trypsin, or hyaluronidase has been shown to improve the initial adhesion of transplanted cells[118] or cartilage-to-cartilage wound edges.[119,120]

Components of the synovial fluid may have an inhibitory effect on integrative cartilage repair. Proteoglycan 4 (PRG4 or lubricin), present in the synovial fluid, normally acting as a lubricant of articular surfaces was shown to strongly reduce cartilage integration.[121]

Integrative cartilage repair appears to be less of a problem for in situ cartilage regeneration (ACI and marrow stimulation) as compared with mature tissue transplantation (OAT or mature tissue-engineered constructs). However, cell-based cartilage repair does need improvement of integrative repair if we plan to extend the indications for cartilage repair, eg, with defect treatment in older patients or in more degenerative joints. Integration of in situ induced neocartilage with older and thus lower cell density cartilage or degenerated cartilage wound edges probably benefits from the above-mentioned interventions.

Outcome parameters

Long-term follow-up of patients is required in order to determine whether cartilage defect repair prevents patients from OA development, as this can take many years. Clinical outcome scores and histological grading of biopsies (in the currently available RCTs often from a small part of the study subjects) are now used to follow the repair process. Methods that are both objective and noninvasive with the ability to follow the repair process and/or the development and progression of OA in time would be of large clinical value. Currently, dGEMRIC, in which a gadolinium-containing contrast agent (Gd-DPTA^2) is injected intravenously followed by MRI, is evolving as a noninvasive method to provide information about the quality of cartilage and repair tissue.[122,123] In damaged cartilage, the GAG content and thereby charge is different compared with healthy cartilage, which also affects mechanical properties.[124] These charge changes are detectable using dGEMRIC as a consequence of differences in Gd-DPTA^2 uptake in the cartilage.[122,125] Recently, dGEMRIC has been studied for follow-up of patients treated with ACI or ACI-derived treatments.[126–128] For ACI-P-treated patients at 9–18 years after treatment, the quality of repair tissue was found to be comparable with surrounding native cartilage; however, no correlation between dGEMRIC results and KOOS was found.[128] Studies using dGEMRIC for evaluation of ACI or ACI-derived treatments are so far conducted in small study populations (5–36 patients).[126–128] To our knowledge, there is only one animal study from which it is concluded that dGEMRIC might be useful for microfracture follow-up.[129] It is evident that larger studies and RCTs are required in order to truly assess the potential of dGEMRIC in follow-up of treated cartilage defects and the early detection of development and progression of OA.

Bioactive materials to improve intrinsic healing capacity

Earlier in this review, we described the use of augmentation of the microfracture technique by using biomaterials. Stimulation of the body's intrinsic healing capacity by the use of bioactive biomaterials is attractive because it can yield an off-the-shelf product. Research focuses on the attraction of cells from the environment (bone marrow, synovium, or even the cartilage) into a scaffold material and to stimulate these cells to form cartilage matrix. Many different types of biomaterials, both synthetic and natural, are being developed, modified, and evaluated. These biomaterials can be made bioactive by incorporation of growth factors or gene vectors to improve cell ingrowth, cell proliferation, or matrix production. Even anti-inflammatory factors can be incorporated to inhibit inflammatory processes, which are known to have negative effects on cartilage repair. Controlled release of these factors in time and may be even sequential release of a number of factors will be necessary for optimal control of the tissue repair process. Research in this area can be expected to reveal new suitable products to improve cartilage repair in the future.

Regulatory obstacles

Stringent regulatory requirements by the US Food and Drug Administration and the European Advanced Therapy Medicinal Therapy regulations have made industrial development of cell therapeutic applications more difficult. Therefore, simpler, and cheaper, single-stage methods, where cell culture is avoided, are receiving more and more attention. Examples of these single-stage methods are the earlier mentioned bone marrow stimulation techniques augmented with biomaterials and the cartilage autograft implantation system (dePuy Mitek Inc., Raynham, MA) where cartilage is minced, added to a synthetic scaffold, and fixed cartilage defects with resorbable staples. More developments are to be expected such as INSTRUCT, a cartilage repair method that is currently undergoing a pilot study in patients where the construct is prepared in the operation theater by combining isolated primary chondrocytes with freshly isolated bone marrow cells seeded in a mechanically functional scaffold (CellCoTEc, Bilthoven, The Netherlands).

Conclusion and future directions

The intrinsic repair capacity of articular cartilage defects is limited, and we believe that these lesions contribute to the development of early OA. The goals of cartilage defect repair should always be a combination of symptom relief and prevention of early joint degeneration. From natural outcome studies, we have learned that it may take many years for isolated cartilage lesions to lead to degenerative changes. However, most cartilage lesions are associated with more extensive joint injuries, contributing to the risk for OA development. For this reason, results from RCTs, with selected patients, cannot fully elucidate the value of current cartilage repair techniques for often more extensive injuries. The above-presented RCTs show an improvement of symptoms following use of current repair techniques with an advantage for cell-based techniques, but they only suggest that the risk for joint degeneration can be reduced.

Successful repair techniques for isolated cartilage defects in otherwise healthy joints may, in the future, be translated to treatment of more extensively degenerated joint diseases such as OA. In degenerative joints, repair of cartilage lesions alone is probably not enough to restore joint function. Because of the progressive nature of OA and the involvement of many different tissues in the joint, this new repair tissue will probably be degraded by a combination of catabolic synovial factors and the altered subchondral bone lying underneath the repaired cartilage. It will, therefore, be necessary to develop a combination of therapies to modulate the degenerative processes, either surgically or pharmacologically, before or at the time of application of a cartilage repair technique.

MSCs can be an attractive cell source for cartilage repair, not only because they are easily harvested, have a good proliferative capacity, and can differentiate into chondrocytes but also because of their trophic activity. MSCs have been shown to secrete a large spectrum of bioactive molecules resulting in a regenerative microenvironment potentially limiting damage and stimulating intrinsic regenerative responses.

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Disclosure

The authors report no conflicts of interest in this work.

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