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Review

Knee chondral injuries: Clinical treatment strategies and experimental models

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ABSTRACT

Articular cartilage has a very limited capacity to repair and as such premature joint degeneration is often the end point of articular injuries. Patients with chondral injury have asymptomatic periods followed by others in which discomfort or pain is bearable. The repair of focal cartilage injuries requires a precise diagnosis, a completed knee evaluation to give the correct indication for surgery proportional to the damage and adapted to each patient. Many of the surgical techniques currently performed involve biotechnology. The future of cartilage repair should be based on an accurate diagnosis using new MRI techniques. Clinical studies would allow us to establish the correct indications and surgical techniques implanting biocompatible and biodegradable matrices with or without stem cells and growth factors. Arthroscopic techniques with the design of new instruments can facilitate repair of patella and tibial plateau lesions.

Contents

Introduction

Joint cartilage is extremely vulnerable to injury, and has a very limited capacity to repair by fibrocartilage. Usually the end point of articular injuries is premature joint degeneration. Injuries to the

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joint cartilage involve loss of macromolecules, rupture of cartilaginous matrix, and finally rupture of the bone matrix; these represent three stages along the same process, which should be borne in mind when treatment is being considered.[1] Patients with chondral injury have asymptomatic periods followed by others in which discomfort or pain is bearable. However, there are no long-term follow-up studies which compare the results of treatment with the natural history of the process. It is also not known how long the repaired tissue lasts, or what the cost–benefit ratio is. Many of the operations performed at present involving biotechnology are associated with high cost and high morbidity, and have not been shown to offer better long-term results.[2]

Aroen et al.[3] analysed 1005 knee arthroscopies performed in three hospitals over a 6-month period. The preoperative radiographs showed joints with signs of degeneration in 13% of cases, chondral pathology of various types in 66% of cases and a chondral defect in 20% of the knees, whilst injuries of International Cartilage Research Society (ICRS) classification[4] grades 3 and 4 were present in 11%. Of all the knees, 6% had injuries greater than 2 cm^2 in size. In another study, Curl et al.[5] reviewed 31,000 knee arthroscopies and found chondral injuries in 63% of patients, whilst 5% of patients aged under 40 had an Outerbridge score of IV in the inner femoral condyle. Hjelle et al.[6] conducted a prospective review of 1000 arthroscopies, and noted that 61% of the patients had cartilage injury measuring 2 cm^2 on average. Widuchowski et al.[7] analysed retrospectively 25,124 arthroscopies and found chondral injuries in 60% of patients; which were classified as chondral or osteochondral injuries in 68%, osteoarthritis in 29%, osteochondritis dissecans in 2% and other types in 1%. Of these, 30% were isolated injuries and the others were associated with damage to other structures. The most frequent sites being affected were the patellar surface and the medial femoral condyle.[7]

The majority of studies of joint cartilage injury and repair have been conducted in adults, but in reality, a small number of cases of articular injuries do occur in children and adolescents, particularly those involved in competitive sports. The articular cartilage injuries in adolescents are usually quite readily categorised as either acute traumatic injury or as osteochondritis dissecans.[8] The articular cartilage of the child is structurally similar to the physis, with zones of provisional calcification. A high proportion of these injuries go undiagnosed initially, resulting in a chondral defect.[8]

Indications for treatment

Lesion size, activity level and patient age are the factors that are generally taken into account to determine the technique for repairing the cartilage. Various treatment protocols have been proposed. One of the best known and most widely used algorithms is based on the size of the injury and the amount of activity carried out by the patient.[9] However, no relationship has been established between the size of the injury and clinical progress after treatment.[10–12] The patient's activity should have an influence on the result,[13] and as such improvements in terms of function should be greater in active patients than in sedentary ones. Other factors for consideration include age, time over which the condition has developed, and the site and depth of the injury. Age could be related to the degenerative process around the injury, or could be a factor, which has a negative impact on the joint itself.[14] The younger the patients are, the better the results tend to be; the best results are obtained in patients aged under 30.[2,13,16,17] A relation also has been observed between the result and the time each patient has to wait from the onset of symptoms until surgery.[10,18] These findings could be related to cell and tissue ageing, which is linked to the regenerative response.

Some secondary factors should also not be ignored, such as the integrity of the joint, that is, the state of the menisci or the ligaments, the weight of the patient and the alignment of the lower limb. According to Cole et al.,[9] the two factors which influence the result of ACI (autologous chondrocyte implant) are age and worker compensation injuries. However, poor alignment of the lower limb, the state of the menisci, the degree of articular stability and the body mass index are also important. A body mass index of <30 is associated with better results.[19] One further aspect is the site of the injury, since injury to the patella is not the same as injury to the tibial plateau or the femoral condyles. The location of injuries treated with ACI has a clear bearing on the clinical results; lesions to the medial femoral condyle are generally found to have better results than those to the lateral condyle three years after surgery[10]; this was also observed in a retrospective study, in which the clinical result in cases of injuries in the femoral condyles treated with microfractures was better than when the injuries were located in the patella or tibial plateau[20] (Fig. 1).

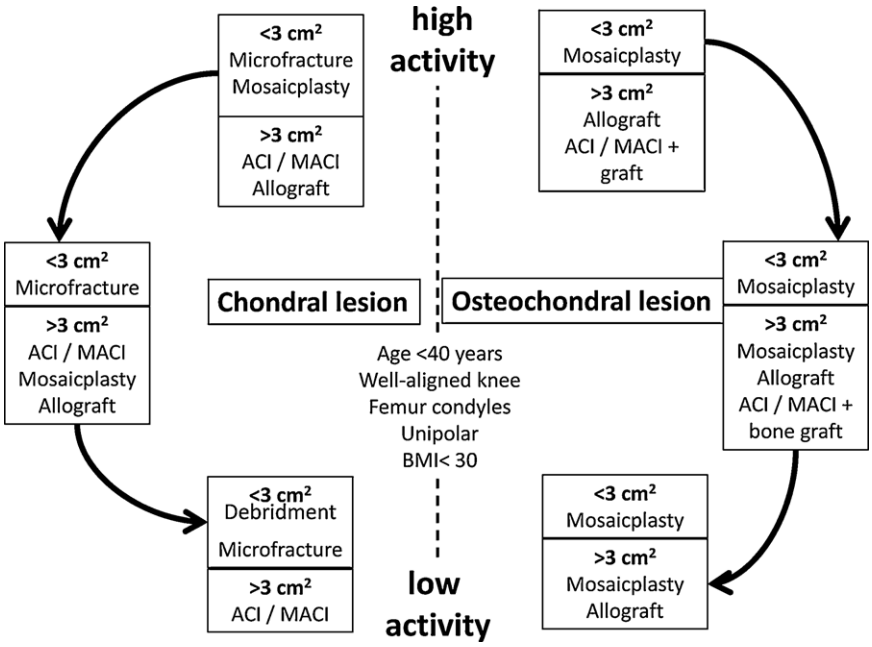

Fig. 1. Algorithm for chondral and osteochondral knee injuries related with size and patient activity.

Table 1
Prospective studies comparing different techniques of treatment of full thickness focal articular cartilage defects.

Authors, citation, year	*N* cases Follow-up	Evidence level	Results	Comments
Bentley et al.[45] 2003	58 ACI 42 OAT 19 months	Level I	Higher Cincinnati score with ACI. Only differences in medial femoral condyle lesions	Arthrotomy Superior histologic results (ICRS) at 1 year in ACI
Horas et al.[46], 2003	20 ACI 20 OAT 2 years	Level II	Similar results with Tegner or Meyers scores. Significant higher Lysholm score in mosaicplasty	Arthrotomy and multiple sizes plugs Mainly fibrocartilage in ACI group
Knutsen et al.[13], 2004	40 MFx 40 ACI 2 years	Level I	Significantly better SF-36 in Micro-Fx. No differences in Lysholm or VAS scales	Higher percentage of hyaline-like tissue in ACI
Gudas et al.[15], 2005	29 MFx 28 OAT 3 years	Level I	96% good and excellent results (HSS and ICRS) in OAT and 52% in MFx	Athletes Younger patients better results
Bartlett et al.[65], 2005	44 ACI 47 MACI 1 year	Level I	Similar Cincinnati score	Similar ICRS score in biopsies
Barber and Iwasko[25], 2006	30 mechanical shaving 30 monopolar radiofrequency probe 2 years	Level II	Both groups improved in Tegner, Cincinnati, IKDC and Vas scales. No difference between groups	No necrosis developed
Knutsen et al.[71], 2007	40 MFx 40 ACI 5 years	Level I	No significant difference No correlation between clinical and histological outcomes	Younger patients did better 30% early signs of OA
Saris et al.[14], 2008	61 MFx −57 ACI+ 18 months	Level I	Similar KOOS scores but less pain in ACI	Better histological results in ACI
Saris et al.[18], 2009	61 MFx 57 ACI+ 3 years	Level I	Significantly better KOOS results	Time to treatment correlates with better results
Gudas et al.[52], 2009	25 OAT 22 MFx 4 years	Level I	MFx deteriorates with time (83% versus 63% good-excellent results after 4 years) ICRS score	Pediatric population Osteochondritis dissecans in femoral condyles
van Assche et al.[39], 2010	33 MFx 34 ACI+ 2 years	Level II	Similar results in functional performance	Slower recovery in ACI group at 1 year
Basad et al.[72], 2010	20 MFx 40 MACI 2 years	Level I	MACI significantly more effective over time according to Lysholm, Tegner and ICRS scores	

MFx: microfracture; OAT: osteochondral autograft transfer; ACI: autologous chondrocyte implantation; ACI+: characterised chondrocyte implantation; MACI: matrix-induced autologous chondrocyte implantation.

Systematic evaluation of surgical techniques

Many techniques have been described for treating injured cartilage, which we can classify into repair, reconstruction and regeneration techniques.[21] The repair methods (perforations and microfractures) help to form fibrocartilaginous tissue, thereby facilitating the access of blood vessels and of osteoprogenitor cells that are capable of achieving chondrogenesis. Reconstruction methods are intended to fill the injury with autologous articular cartilage transplantation or allografts (OATS or osteochondral autograft transfer, mosaicplasty, allografts) by arthroscopy or a mini-arthrotomy approach. Finally, regenerative methods make use of bioengineering techniques to develop hyaline cartilage tissue (autologous chondrocyte implant (ACI), mesenchymal stem cells (MSCs) or chondrocytes in different scaffolds (MACI))[21] (Table 1).

Repair techniques

Arthroscopic debridement

The role of arthroscopic lavage and debridement remains controversial and the principal indication is the treatment of concurrent meniscal tears in patients with minimal malaligment.[22] However most of the studies evaluating this procedure results are performed in degenerative lesions. The results are mediocre, and there is no evidence that it has an effect on outcome or cartilage repair.[23] The cleaning process may relieve symptoms, but with temporary effects. The results are better in the case of unstable cartilage.[24] Debridement using monopolar electrodes is more regular and more shallow than motorised debridement, although the thermal effects are not yet fully understood, and appear to depend heavily on the type of electrode used.[25]

Stimulation of the bone marrow

These systems are intended to stimulate cell migration and cytokine expression to repair the cartilage.[26] They include Pridie perforations with Kirschner's wires,[27] abrasion using a burr as far as the bleeding subchondral bone, and microfractures.[17,28–30] These techniques are the methods of choice for orthopaedic surgeons, as they are straightforward, fast and inexpensive.[31] Nonetheless, the cartilage is usually repaired with fibrous tissue or collagen type I fibrocartilage, because the number of chondroprogenitor cells is too small to achieve tissue regeneration[32] or too slow, giving rise to subsequent degeneration of the tissue.

Microfractures are the gold standard for the treatment of chondral lesions, and serve as a point of reference for comparing all other techniques. The perforations are made using an angulated icepick to cross the subchondral bone to provoke bleeding and gain access to the bone marrow, thereby avoiding the thermal damage involved in power drilling. Bleeding brings about a greater surface and quality of repair tissue, stimulating the haematopoietic and MSC to form new tissue, even though the blood partly clots and

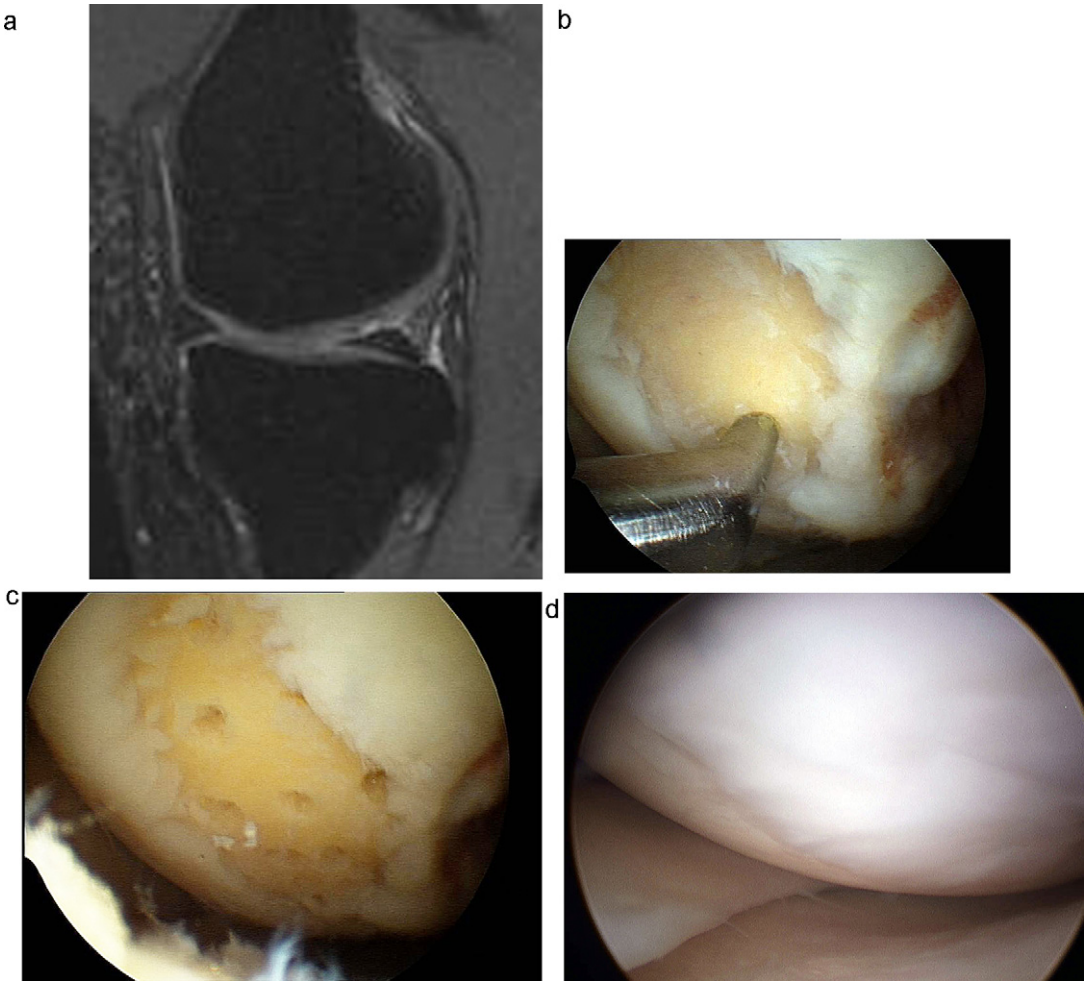

Fig. 2. Microfracture in a chondral lesion, (a) MRI pre-op, (b) arthroscopic view, (c) microfractures, and (d) second look one year after operation.

most disappears with the arthroscopic lavage and synovial fluid dilution. Despite the criticism it has received, no other technique is so widely used today. Microfractures are effective in small injuries (less than 2 cm^2) with an intact subchondral plate.[17,29] They should be performed with appropriate icepick, creating stable, vertical edges; the calcified layer of the cartilage must be debrided, and perforations made every 2 or 3 mm to ensure good anchorage of the clot. A physiotherapy protocol should be followed with intense passive mobilisation.[33] No results have been published concerning the use of this technique in children (Fig. 2).

To maintain the blood clot and the bone marrow cells in the lesion, scaffold guided regenerative medicine SGRM has been proposed, which applies chitosan, a natural polymer scaffold, as a patch which is reabsorbed, leaving all the blood products and MSCs in the area of the injury, protected from the synovial fluid, in order to form hyaline cartilage.[34,35]

Clinical evaluation of microfractures

Kreuz et al.[20] concluded that microfractures show signs of deterioration from 18 months after surgery onwards, and that the best prognosis is in patients aged less than 40 years, with injuries in the medial femoral condyle. The review carried out by Mithoefer et al.[19,33] shows that microfractures have excellent results in the short term, but that long-term studies are lacking. This technique has been criticised for providing only limited repair to the hyaline cartilage, with poor production of repair tissue and the possibility of future deterioration over time.

Cerynik et al.[36] analysed the progress of cases of microfractures in chondral injuries in 24 NBA basketball players, comparing the efficiency of the players two years before and after treatment. The mean time elapsing from the time of the operation until a player returned to competitive sport was 30 weeks. In the first season after the operation, the player's effectiveness and the number of minutes he played both dropped. The 17 players who played for two or more seasons again lost effectiveness and minutes during the second year. Nonetheless, despite the importance of this study, we have no knowledge of how these players would have progressed had other techniques been used, or had their injuries been left untreated. Three years after surgery, de Windt et al.[10] found no differences in the clinical assessment (KOOS) of patients who had received microfractures and those who had ACI. Moreover, they concluded that the size of the defect did not influence the outcome. Solheim et al.[37] compared the results of microfractures in 110 patients with a mean age of 38 years (range: 15–60 years), divided into two groups, one with single chondral lesions, the other with multiple injuries. The Lysholm scale was used to assess the results. They found 24 failures (22%), 18% of the group with a single injury and 29% in the multiple-injury group. The pain was less and the functional evaluation better in the group with a single injury. Steadman et al.[17] and Mithoefer et al.[38] found that the return to sporting activities after microfractures was achieved to better effect when the patients had undergone symptoms for less than one year, the injury was less than 2 cm^2 in size, and the patients were aged under 40. van Assche et al.[39] followed patients operated on with ACI for two years, and

compared the results with those treated with microfractures. The 67 patients had a mean injury size of 2.4 cm^2 in the femoral condyle. Microfractures were performed on 33 patients and ACI on 34; both groups followed the same physiotherapy protocol. After 2 years, the patients treated with ACI had functional results that were very similar to those operated on using microfractures. In a randomised study, Knutsen et al.[13] compared microfractures with ACI, and found that there was an improvement of the SF-36 score in cases where microfractures were used, but detected no significant differences on other functional or histological scales after 2 years.

Substitution techniques

Autologous osteochondral grafts (mosaicplasty)

Transplantation of the bone-hyaline cartilage composite has been proposed as a method for replacing the subchondral bone when it is affected (osteochondritis dissecans) and for providing more long-lasting hyaline cartilage. Osteochondral autograft provides a structure that integrates easily with the surrounding bone, although the cells on the edge of the cylinder die, which compromises its integration.[40] For this reason, the graft should be inserted press-fit so that there is a good contact with the healthy tissue (Fig. 3). To guarantee the optimum results, a single cylinder should be inserted with a maximum diameter of 12 mm. In general, several cylinders 4–10 mm in diameter are used, which must be inserted using a specific delivery tool, strictly perpendicular to the surface, maintaining total contact and the congruence of the joint.[41,42] Grafts should be inserted in the periphery first to provide support for central grafts. The depth at which they are inserted on the bone bed must be at least the same as the length of the graft that will be exposed.[43] The spaces that are left between the cylinders will never be filled with cartilage. Furthermore, each cylinder should be inserted individually in its own hole, but care must be taken not to produce an impact, since this will damage the joint cartilage and reduce the viability of the cells.[44] The percentage of excellent and good results is greater when this technique is used in the condyles rather than in the tibial plateau or in the patella.[45]

Mosaicplasty is associated with technical difficulties which explains why it is used less than other techniques despite the good clinical results that have been obtained, although these results appear to vary greatly from one team to another.[45–47] These difficulties include the lack of available tissue, morbidity at the donor site, and the difference in cartilage thickness between the donor and host sites.[48] Also in crater shaped defects, restoring a congruent bevel to the convex condylar surface is a challenge when bone loss is greater than 8 mm. The grafts in the centre of the defect need to be more prominent than those at the periphery and will consequently have a longer length, as unsupported grafts tend to subside, becoming covered in fibrous tissue.[49]

Clinical evaluation of mosaicplasty

Hangody and Füles[47] report the results in 831 patients, which were good or excellent in 90% of cases when performed in the talus and the femoral condyle, and in a slightly smaller percentage of those performed in the tibia and patella. Complications were reported in 3% of operations, with 4 deep infections and 36 painful postoperative hemarthrosis. Jakob et al.,[43] in a series of 52 patients, 23 with an injury of ICRS grade 3 and 20 with a type 4 injury, with mean follow-up of 37 months, reported excellent knee function in 86% of cases, and identified a relationship between the complications that ensued and the size of the lesion. Horas et al.[46] in 40 patients with a femoral condyle injury, performed a prospective and randomised study comparing ACI and mosaicplasty patients group. After two years, the functional results were similar in the two groups, although the Lysholm score was better after mosaicplasty. Histologically, in cases of ACI they mainly found fibrocartilage. In the comparative, randomised multicentre study by Dozin et al.,[50] one third of the enrolled patients improved just with a previous debridement. In the 23 patients left, there was a complete recovery (Lysholm Knee Scoring) in 88% of the patients treated with mosaicplasty and 68% of those treated with ACI. However, Bentley et al.,[45] in a randomised study, patients with ACI were found to have better clinical and histological results one year after the operation than those treated with mosaicplasty. These differences were only statistically significant in the case of injuries to the medial femoral condyle. They also observed that all the

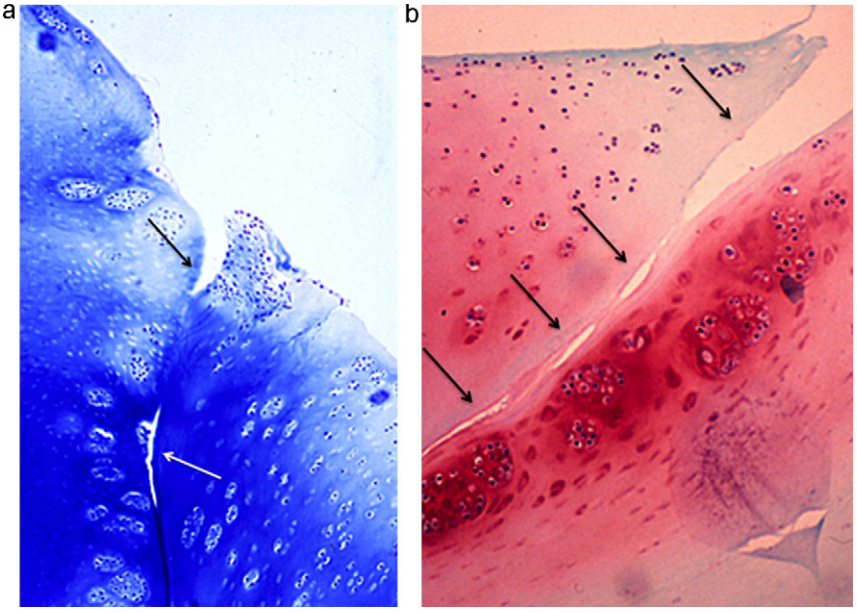

Fig. 3. Experimental model of osteochondral allograft plugs in sheep. If the contact between the plug cartilage and the host cartilage are not perfect, they will not be able to heal ((a) Masson trichrome 10×, (b) Safranine-O, 10×).

patellar mosaicplasties failed. Gudas et al.[51] also performed a randomised clinical study to compare mosaicplasty and microfractures in 57 athletes with a mean age of 24 years who had symptomatic lesions to the knee joint. Of the patients treated with mosaicplasty, 96% had good or excellent results, compared with 52% of those treated with microfractures. In the course of follow-up, the mosaicplasty group was also found to have better results than the microfractures group; 93% of the mosaicplasty group and 52% of the microfractures group returned to their sporting activities at the same level of intensity as before surgery within 6 months. Gudas et al.[52] also compared the results of osteochondritis treated with mosaicplasty and microfractures in young people aged under 18. After one year, both groups had improved, but after 4 years, they observed a deterioration in the microfractures group.

Osteochondral allografts

The advantage of allografts is that they are adaptable, as grafts can be designed for lesions of any shape or size, and they can be obtained from weight-bearing areas so that they are identical in form and curvature to the injured area. Moreover, they can be harvested without endangering the donor site. The disadvantage is that they have to be used in a short period of time, since they must be kept fresh in serum, and this is only possible for a few weeks after extraction, because cryopreserved cartilage is a matrix with few viable cells, and this affects the recovery of the cartilage morphology. There is also a risk of immune reactions and the transmission of disease. A major reduction in cell viability has been observed when the implants are kept in culture for more than 72–96 h, which severely limits the uses of this technique.[53]

Allografts are indicated in patients aged up to 50 years, with injuries greater than 2.5 cm in diameter and major bone loss. Several previous studies suggest chondrocyte viability at the time of implantation is an important factor in ensuring long-term osteochondral allograft survival in vivo.[54] The literature reports survival in 85% of cases after 10 years and 74% after 15 years in the femoral condyles, but the percentages are lower in the tibial plateau. The results are better in young, active patients.[55] For Micheli et al.,[56] in cases of OCD, which is typical in adolescents, this technique offers an advantage over ACI, then resolved the necrosis of the subchondral bone.

Synthetic plugs

Biphasic cylindrical scaffolds made of synthetic co-polymers greatly facilitate the techniques for filling osteochondral defects. The plug is designed to provide the benefits of marrow stimulation together with structural support to allow regeneration of articular cartilage to the same height as that of the surrounding articular surface. The advantage is that the right thickness and length can be chosen to fit the dimensions of the gap. In addition, such plugs can be combined with stem cells or growth factors. Nonetheless, despite the short surgical time involved, it would seem that they cannot yet be used to replace auto- and allografts, since cases have been described in which pain or persistent swelling have occurred, where the plug has not been completely incorporated.[57]

Regeneration techniques (ACI/MACI)

This technique is currently proposed as a second line of treatment for when the abovementioned techniques fail, in injuries of 2–10 cm^2 in well-aligned knees in young, active people. In the mid-1990s, one of the first tissue bioengineering techniques was proposed (ACI), and in the last 15 years over 12,000 such operations have been performed.[58] On the basis of a hyaline cartilage biopsy, the cartilaginous tissue obtained is digested, cultivated, the cells are expanded and subsequently implanted in the patient after a period of time. Once an adequate quantity of cells has been obtained in a second operation consisting of arthrotomy, which can be performed after 6 weeks, the lesion is cleaned, taking special care not to damage the subchondral bone, and the cultured chondrocytes are deposited on the bone and covered with a periosteal membrane which is sutured to the healthy cartilage and sealed with fibrin.[59] In the case of deep osteochondral lesions, Brittberg et al.[60] proposed filling the defect with a bone graft and inserting a membrane on which the cells were deposited, which was then covered with another membrane, sutured and sealed; this technique was known as the ACI "sandwich".

The first generation ACIs were associated with complications and problems with cartilage hypertrophy and ossifications resulting from the use of periosteum. Subsequently, the second generation known as MACI introduced seeded membranes and biomaterials such as type I collagen,[61] a matrix based on hyaluronic acid[62] and type I/III collagen.[63] When this type of membranes were used, the hypertrophy was reduced to 5% of all cases, and the problems disappeared 3–6 months after the operation when the membrane was reabsorbed.[63] Two studies compared first-generation ACI with the second-generation type, but found no clinical differences between them.[64,65] When regeneration is effected using second-generation implants, large areas of fibrocartilage are also found, possibly because of their low cellular density and poor proliferative capacity.[46] Moreover, in this technique healthy cartilage is always sacrificed in order to regularise the lesion.

Third-generation techniques, which are very limited at the present time, have been devised with a view to improving the earlier methods. These propose chondroinductive or chondroconductive matrices with allogenic cells and techniques to improve the mechanical conditions so that a suitable tissue can be developed before surgery.[66] Fourth-generation techniques have also been proposed,[67] which are based on polymers such as elastin, or hydrogels, to obtain a homogeneous three-dimensional distribution of the cells. Gene therapy with non-viral genes has also been included, so that the stem cells express the desired growth factors.

Clinical evaluation of ACI and MACI

Peterson et al.[68] published the results they obtained in their first 100 patients, beginning in 1994, with follow-up ranging from 2 to 9 years. Of these, 92% had an isolated femoral injury, and 89% were found to have osteochondral defects, but satisfactory results were obtained. Furthermore, 30 out of 31 patients expressed the same degree of satisfaction with the technique 7 years later. In the general clinical evaluation, 80% of the results were excellent and good after 2 years. When a second arthroscopic assessment was performed, no fibrillation of the regenerated tissue was detected. Later, Brittberg,[69] in a review of 244 patients treated with ACI followed for 2–10 years, obtained 84 and 90% of good and excellent results respectively in cases with an isolated injury to the femoral condyle, whilst this percentage fell to 74% when other locations were involved.

Zaslav et al.[12] studied the progress of ACI in patients who had previously undergone surgery with medullary stimulation techniques, perforations or microfractures, and found no differences regarding function and pain after ACI; however, Minas et al.[70] showed that patients who had previously been treated with abrasion, perforations and microfractures had three fold of failures when autologous chondrocytes were implanted when compared with patients who had not been treated previously.

If we restrict our enquiry to the Level I studies, Bentley et al.[45] found 88% good and excellent functional results when ACI was used, compared with 69% with mosaicplasty, over a mean follow-up period of 19 months. The arthroscopic results after one year were also significantly better when ACI was used. As mentioned before, Knutsen et al.,[13] in 40 patients, found no significant functional or histological differences after two years between microfractures and ACI techniques. At five years the results remain similar but early degenerative signs were present in 30% of the patients.[71] Saris et al.[18] compared microfractures with ACI using a particular cell therapy (ChondroCelect, TiGenix NV, Lovaina, Bélgica) 36 months after the operation, in 118 randomised patients aged between 18 and 50 with injuries of grades 3/4 on the ICRS score. The overall KOOS score was similar in both groups, although the results related to pain and quality of life were significantly better in the ACI group. The histomorphometric assessment was also superior in cases where ACI had been used, and the reaction of the subchondral bone worsened significantly over time in the cases of microfractures in comparison with those that had received ACI. At a longer follow-up, KOOS scores became superior in ACI group and they noticed that a short time to treatment correlated with better clinical results.[18] Basad et al.[72] in a two years prospective and randomised study, reported MACI functional results to be better than microfractures using different scores. Finally, Bartlett et al.[65] carried out a prospective randomised study comparing MACI and ACI covered with a membrane derived from porcine type I/III collagen (ACI-C) in 91 patients. Both groups were found to have similar results two years after the implant and both treatments brought about a clinical improvement one year later. The index of hypertrophy in the ACI-C group was 9% (4/44), and 6% (3/47) in the MACI group and in both groups, 9% of the patients required further surgery.

The innovative aspects of this technique have attracted considerable attention in the literature, with over 800 articles in Medline.[2] There are multi-centre studies, like that of Micheli et al.[8] which found 94% implant survival in 3-year follow-up of 50 patients with a mean age of 36 years and an average defect of 4.2 cm^2. The Cartilage Repair Registry,[73] showed that 78% of the injuries treated with ACI improved, and that the objectives were met in 81% cases of single lesion to the femur. Finally, Moseley et al.[74] confirmed that after 10 years, 69% of the procedures had improved, with a failure rate of 17% of cases and 12.5% that presented no changes since the operation.

As far as MACI techniques are concerned, Marcacci et al.[62] analysed the Hyalograft-C© in a multi-centre study of 175 patients in whom 216 chondral defects of the knee were repaired using an open approach and followed for an average of 2 years. Of these patients, 93% improved on the IKDC scale; those with traumatic lesions or osteochondritis dissecans had better results than those with microlesions and degenerative injuries; 88% had normal or almost normal IKDC scores. After 2 or 3 years, 102 patients were assessed again, and the IKDC scores were found not to have fallen.

No MACI technique has been shown to have clinical or histological superiority to ACI[75] since even though the scaffolds simplify the surgical technique, they do not achieve homogeneous distribution or sufficient chondrocyte density to promote cell differentiation and formation of the cartilaginous matrix.

Experimental studies on cartilage repair

Articular cartilage basic research is the way to achieve better clinical results by the application of tissue engineering techniques. Experimental studies of cartilage repair produce different results depending on the animal used, the type of lesion, and the techniques applied for the study. Animals range from mice, through rabbits, to sheep, goats and pigs. There are few studies in dogs and horses. The small and medium-sized animals are quadrupeds, that is, in no case do they have to bear loads like the human being, and their cartilage is thinner than that of humans. On the other hand, horses are hard to manage, and their cost makes them difficult to use. Rabbits are of an appropriate size for surgery, and are easy to maintain for a reasonable period of time. Moreover, these experiments are reproducible and relatively economical. Rats, on the other hand, are too small for surgical manipulation, even though they are frequently used in gene therapy, because their small size means that fewer vectors are needed, and costs can therefore be reduced. Dogs are rarely used because of their cost and social connotations, which means that the choice is usually between sheep, goats or pigs. These animals have larger joints than the rabbit, can sometimes be operated on in both legs, and have thicker cartilage in their joint surfaces. This means that more cartilage can be harvested in order to culture chondrocytes, larger lesions can be effected and different studies can be carried out.

Donor age is an important factor in determining the outcome and potential success when tissue-engineered cartilage is produced from articular chondrocytes.[76] More specifically, primary chondrocytes from aged donors may not possess sufficient capacity to produce the extracellular matrix that is required for a mechanically resilient tissue. Acosta et al.,[77,78] reported that osteoarthritic cells showed a poor response according to matrix gene expression, whilst young cells responded properly, and aged chondrocytes showed a moderate response. These results suggest that the state of cartilage may affect the behaviour of cultured chondrocytes and, on the other hand, discourage the use of frozen cartilage because of the decrease in cell viability and elevation in metaloproteinases.

Heterotopic models (nude mice models) have been used for studying chondrogenesis in vivo, but in experimental orthopaedic surgery, the most usual models focus on osteochondral defects. The type of lesion is also different, although in this the studies are more homogeneous. Experimental models use areas of load in the weight bearing areas of the knee (femoral condyle), since the area is larger and easier to reach, or the femoral trochlea, an area which, though free of load, suffers maltracking of the patella. There are few studies in joints other than the knee, since this joint is easy to access and larger in size than the other options. The main interest lies in the model of lesion that is used, and the repair technique. We can distinguish chondral lesions which do not affect the subchondral bone, osteochondral lesions which affect the cartilage, subchondral bone and trabecular bone of the metaphysis, and finally, arthritic degenerative lesions, although these fall outside the scope of this article.

Nonetheless, it is far from simple to evaluate the results of experimental studies, and the criteria vary from one study to another. Studies that are done with cartilage repair are generally macroscopic studies to describe the appearance of the repaired tissue, histology and histomorphometry, in order to evaluate the quantity and quality of the repaired tissue. For this purpose, there are various different systems for evaluation and modification.[79–81] There is now greater consensus concerning the ICRS and OARSI scores, since these are more complete, and more complex.[82,83] However, these scores are subjective in many cases, and do not help to define the degree of repair properly. These studies can be complemented by electronic microscopy and MRI to assess cartilage and subchondral bone repair. Metabolic activity is determined by $_{35}SO_4$ uptake, and viability is assessed using a live/dead stain and by confocal laser microscopy. Bromodeoxyuridine (BrdU) positive, proliferating, cells are enumerated and localised. Mechanical assays of joint cartilage and studies of the repair show how far these tissues have the capacity to resist load. These are usually performed by indentation or pressure tests.

Experimental studies using microfractures

Microfractures are used as the reference group or gold standard in many studies. Bone marrow stimulation is performed using several surgical techniques that have not been systematically compared or optimised for a desired cartilage repair outcome.[35,83] Chen et al.[35] noted differences between microfracture and drilling for acute subchondral bone structure and osteocyte necrosis. Chevrier et al.[84] in rabbit found that in the microfractures covered with chitosan–glycerol phosphate (GP) chondrogenic foci appeared, and had some similarities to growth cartilage. These could give rise to a repair tissue that has similar zonal stratification to articular cartilage. For Hoemann et al.[85] macrophages may play a role in the regeneration of subchondral bone, and chitosan–GP can attract and transiently accumulate these cells in the repair tissue. The technique improved subchondral repair and could be advantageous for enhancing integration of a restored chondral surface to the subchondral bone. Similar results were obtained[86] in the knee joint of minipigs, where microfractures were left empty or covered with a collagen membrane, or treated by MACI. Significant outgrowths of subchondral bone and excessive endochondral ossification within the repair tissue were regularly observed in microfractures. In contrast, such excessive bone formation was significantly inhibited by the additional transplantation of chondrocytes.

Experimental studies using different scaffolds

An increasing number of studies are currently focusing on membranes or structures which permit the inclusion of expanded cells or growth factors. Membranes have become a frequent system for use in experimental studies. Most studies use collagen type I membranes,[87] collagen type II[88,89] or type III, type I/III[90] alone or combined with hyaluronan and fibrin,[91] polyglycolic acid resorbable membranes (PGA), polyethyleneimine,[92] PLGA-gelatin/chondroitin/hyaluronate with TGF-β1,[93] poly-L,D-lactic acid scaffolds alone or seeded with autologous chondrocytes that seems too stiff for cartilage repair,[94] polysulphonic membrane[95] PLLA, composite of polydioxanon/polyglactin, and dry-frozen dura[96]; collagen scaffolds containing calcium phosphate and scaffolds of poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid)[97]; nanocomposite scaffold with hydroxyapatite nanoparticles[98]; rabbit BM-derived MSC with FGF in hyaluronic acid gel sponge.[99] Lin et al.,[100] introduced an injectable composite scaffold for cartilage using allogenous porcine cartilage microparticle acellular tissue matrix and fibrin glue.

The results vary greatly from one study to another. Lee et al.,[101] with a canine model, implanted autologous chondrocyte-seeded type II collagen scaffold and demonstrated that the compressive stiffness of the repair tissue was 20-fold less stiff than the original articular cartilage. For Mimura et al.,[102] collagen scaffold was shown to recruit a significantly larger number of proliferating cells to the central region of the cartilage defect and the histological grading score for the regenerated cartilage treated with collagen gel was superior to that of the other groups. Type II collagen gel is suitable for injection into cartilage defects without any covering of a graft and offers a useful scaffold during chondrocyte transplantation.[89] In another publication, MSC seeded in type I collagen-glycosaminoglycan matrices have been shown to produce a solid cartilaginous tissue containing type II collagen after being cultured in the chondrogenic differentiation medium and implanted into cartilage defects.[100]

Rudert et al.[96] observed no significant differences between the unseeded matrices and the untreated control defects. Kon et al.,[98] in osteochondral defects in sheep, found no difference in cartilage surface repair between cell-seeded and cell-free groups six months after surgery. For these authors, the mode of action of the scaffold is based on the recruitment of local cells. Similarly, Chiang et al.[104] compared the efficacy of cartilage regeneration by in vitro-expanded chondrocytes at high density and freshly harvested chondrocytes at low density in osteochondral defects at the weight-bearing surface of femoral condyles of domestic pigs. At 6 months, according to the ICRS score, cartilage could be regenerated in both groups with comparable quality. The authors concluded that culture of chondrocytes before implantation is not necessary.

Filová et al.[87] seeded the scaffold with autologous chondrocytes, into rabbit femoral trochlea, and found this to be more effective after 6 weeks. The scaffold can therefore enhance cartilage regeneration by supporting the hyaline cartilage formation. Chang et al.[103] in mini-pigs, showed satisfactory results when the repair tissue was hyaline cartilage or fibrocartilage in the tissue engineering-treated group. Willers et al.,[90] in osteochondral lesions using a rabbit model, concluded that ACI with collagen membrane showed significant improvement as compared with untreated controls, and found that cell density had no effect on outcome histology. Köse et al.[97] in collagen and calcium phosphate compounds (CaP-Gelfix) and poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) scaffolds showed cells maintained their phenotype in both matrices, but PHBV had better healing response than CaP-Gelfix.

Experimental studies in osteochondral defects

The problem with osteochondral plugs is the integration into the healthy bone tissue. If the contact between the plug and the cartilage is not perfect, they will not be able to heal.[105] Lane et al.[106] to prevent the occurrence of such a gap, combined microfracture and osteochondral autograft transfer procedures. For treatment of osteochondral lesions, experiments have also been conducted with autografts,[48,106] allografts[105] and synthetic osteochondral plugs. This implants address the malleable properties of cartilage whilst also mimic the rigid characteristics of subchondral bone. Tanaka et al.[107] used a biphasic construct to repair osteochondral defects in articular cartilage: the plugs were made of chondrocytes in collagen gel overlying a resorbable porous beta-tricalcium phosphate block. Frenkel et al.[108] investigated two different multiphase implants in rabbits. The hard portion of both devices consisted of D-L-polylactic acid invested with hyaluronan and the soft superficial part was polyelectrolytic complex hydrogel of hyaluronan and chitosan in the first implant and type I collagen in the second one. Both implants were excellent vehicles for chondrocyte or stem cell transplantation. Chiang et al.[104] used biphasic cylindrical porous plugs of DL-poly-lactide-co-glycolide and beta-tricalcium phosphate and reported that cartilage regenerate in both materials in comparable quality but more interesting was that chondrocytes culture before implantation was not necessary. Other studies focus on MSC incorporated with a poly(L-lactide-co-epsilon-caprolactone) scaffold[109]; or alginate and gelatin hydrogel plus autologous chondrocytes or periosteal cells.[110]

Lane et al.[48,106] in a goat model, found no evidence of gross morphologic or histologic changes in the operated knee as a result of the osteochondral donor or recipient sites. The patella, tibial plateau, and medial meniscus did not show any increased degenerative changes as a result of articulating against the donor or recipient sites of the osteochondral autografts. Biomechanically, 6–7-fold higher stiffness was noted in the cartilage of the transferred plugs compared with the control medial femoral condyle. On the histologic evaluation, the healing subchondral bone interface at the recipient site had increased density. Glycosaminoglycan synthesis was upregulated in the transplanted cartilage plug relative to the contralateral control, showing a repair

response at the site of implantation. Finally, confocal microscopy showed 95% viability of the transferred plugs in the medial femoral condyle region. These findings demonstrate the ability to successfully transfer an osteochondral autograft plug with maintenance of chondrocyte cellular viability.

Experimental studies using ACI

ACI is the experimental technique which has proved most attractive in terms of achieving regeneration of the joint cartilage. Kamarul et al.[111] compared the efficacy of ACI versus non-operative treatment for cartilage repair in rabbits. Jubel et al.[112] in full-thickness cartilage defects of femoral condyles in sheep, found that ACI leads to a qualitatively better regenerative tissue than does periosteal flap alone or no treatment. Boopalan et al.,[113] in a 3 mm defect created in the rabbit knee, transplanted cultured allogenic chondrocytes and closed the injury with a periosteal flap. Cartilage defects treated with chondrocyte transplantation result in better repair tissue formation with hyaline characteristics than those in control knees. Vasara et al.,[114] in a 6 mm deep chondral lesion in the knee joints of immature pigs, compared ACI with the use of a periosteum flap and the control group. At one year, the repair tissue was nearly normal in all cases in the spontaneous repair group and in 38% of animals in the ACI group. The spontaneous repair ability of full thickness cartilage defects of immature pigs is significant, and periosteum or autologous chondrocytes bring no additional benefits to the repair.

However, few studies have been able to demonstrate the regeneration of hyaline cartilage either clinically or in experimental conditions, because simpler techniques have become available which do not require the cells to be expanded in the laboratory, since they involve the use of MSC isolated from bone marrow to generate different cell types and thereby function effectively in tissue repair. MSCs can be harvested from bone marrow by a small puncture of the iliac crest of patients. In contrast to chondrocyte transplantation, this procedure creates no additional harvest defect in the knee joint of the patient. Incorporation of MSCs in suitable tissue engineering scaffolds and culture in chondrogenic medium can produce cartilage-like tissue. Only MSC from bone marrow can be cultured and are able to differentiate to appropriate bone and cartilage cell lines.[115]

Chang et al.[103] investigate autologous uncultured bone marrow-derived mononuclear cells with fibrin gel, autologous uncultured peripheral blood-derived mononuclear cells with fibrin gel, fibrin gel alone, or nothing, transplanted to the articular cavity 7 days after the operation, and concluded that the transplantation of autologous uncultured bone marrow-derived mononuclear cells contributes to articular cartilage repair. Sheep knee joints treated with autologous MSCs cultured in chondrogenic medium showed clear evidence of articular cartilage regeneration in comparison with other groups.[116] Mrugala et al.[117] created, in sheep, a partial-thickness cartilage lesion in the patellae filled with MSC with or without chitosan, with or without TGF-β3, in a fibrin clot. The histological analysis revealed chondrocyte-like cells surrounded by a hyaline-like cartilaginous matrix that was integrated in the host cartilage when MSC were combined with chitosan and TGF-β3. Finally Zhou et al.,[118] showed that implanted MSCs can differentiate into either chondrocytes or osteoblasts and be effective in repairing also articular osteochondral defects.

Experimental studies with growth factors in cartilage

In cartilage tissue, chondrocytes are responsible for synthesising and renewing the matrix that surrounds them with a constant turnover mechanism.[77] Growth factors may enhance current cartilage repair techniques via multiple mechanisms including recruitment of chondrogenic cells (chemotaxis), stimulation of chondrogenic cell proliferation (mitogenesis) and enhancement of cartilage matrix synthesis.[119]

Hurtig et al.[120] showed that sheep knee joints that received rhBMP-7 immediately after an impact injury had small focal lesions at the injury site that did not progress into the surrounding cartilage. Joints that received BMP-7 3 weeks after injury were improved and had limited progression compared to controls, but joints that received the protein 12 weeks after injury had no statistically significant improvement. These studies suggest that BMP-7 may be chondroprotective after traumatic injury in patients if it is administered within 3–4 weeks of the index injury.

Nawata et al.[121] used rHuBMP-2 to repair cartilage defects in rats. Cartilage was not formed in the presence of 1 μg rHuBMP-2 or in the absence of rHuBMP-2 but was observed in defects receiving 10 mg rHuBMP-2, which were repaired and restored to normal morphologic condition within 6 months after surgery. Acosta et al.[77] studied young, aged, and osteoarthritic cartilage cells from sheep, cultured in monolayer. A decrease in expression of type II collagen and aggrecan in aged and osteoarthritic chondrocytes was found. Treatment of cells with growth factors aFGF, IGF-I, TGF-β, and BMP-7, especially the combination of IGF-I and FGF, improved the proliferation rate in all the cells studied and stimulated gene expression of type II collagen, aggrecan, and TGF-β. Singh et al.[122] determined the influence of IGF-1 in cartilage repair with or without autografting and showed in the outcome that cartilage formation apparently declined and appeared to converge to osseous tissue. Mizuta et al.[123] demonstrated, in full-thickness articular defects, that FGF-2 is crucial for the proliferation of pre-chondrogenic mesenchymal cells during chondrogenic induction. Endogenous FGF-2 could not meet the requirements of growth signalling in the centre of larger sized defects. FGF-18 can stimulate repair of damaged cartilage in a setting of rapidly progressive OA in rats.[124]

Nishida et al.[125] found that connective tissue growth factor (CTGF)/CCN2 is a unique growth factor that stimulates the proliferation and differentiation, but not hypertrophy, of articular chondrocytes in vitro, rCTGF/CCN2 enhanced type II collagen and aggrecan mRNA expression in mouse bone marrow-derived stromal cells and induced chondrogenesis in vitro. For Katayama et al.[126] cartilage-derived morphogenetic protein 1 (CDMP1), a member of the TGF-β family, is an essential molecule for the aggregation of mesenchymal cells and acceleration of chondrocyte differentiation.

Experimental studies of cartilage gene therapy

The use of gene therapy strategies, *ex vivo* or in vivo, combining the introduction of genes capable of inhibiting catabolic activity (IL-10 or IL1-Ra) and inducing anabolic activity, could prove effective in the complete regeneration of damaged tissues. The greatest disadvantages of joint therapy using IGF-I are the short average life of the protein in biological systems, and the low response of cells from damaged or old cartilage.[127] The former problem was addressed in earlier studies by repeated administration or the use of fibrin patches capable of releasing the protein gradually.[128] Gene therapy is still at the experimental stage and appears not to have a clinical application actually, although it does allow us to transfect chondrocytes or stem cells with BMP-7 gene,[129–131] BMP-4,[132] BMP-2,[133] IGF-1,[134,135] FGF-2[135,136] or sonic hedgehog (Shh) gene.[131] These cells could be cultured to increase the number of cells and then be seeded onto bioresorbable polymer scaffolds. The utility of tissue-engineering strategies in which gene therapy is used to locally influence the repair environment needs to be demonstrated.

Future directions

Although these procedures have been implemented with varying degrees of success, no consensus exists on the gold-standard of treatment. The best way to address the question regarding which treatment is superior would be a large multi-centre trial comparing all the described techniques.[137] All these techniques improve the symptoms and course of chondral lesions. However, there is no clear evidence to suggest which one is best. The repair of focal cartilage injuries requires a precise diagnosis and a correct indication to provide a surgery proportional to the damage and adapted to each patient, so that the mechanical conditions of the repair match with the morphology of the regenerated tissue. However, it should also be kept in mind that cartilage injuries have a very precise aetiology and it is essential to establish the cause and try to solve each case appropriately.

At present, it is fair to say that all the techniques described here improve the symptoms in the short term after surgery; however, joint cartilage lesions are often first noted in the course of arthroscopy. In such cases, when the injury is smaller than 4 cm^2, the first line repair could be considered by means of perforations or microfractures, as long as the subchondral bone is intact.

The future of cartilage repair should be based on an accurate diagnosis using new MRI techniques for imaging cartilage (dGEMRIC, T2 weighted image) to estimate the size/depth of lesions and assess how they progress. Time will enable us to determine which of the currently available techniques are most reliable. Clinical studies will also allow us to establish the correct indications and improve repair with biocompatible, biodegradable, permeable matrix; although many scaffolds have been found to be effective, it remains to be determined which have the best mechanical characteristics and cost–benefit ratio. It appears that bone marrow aspirate of MSC may differentiate towards chondrocytes or osteoblasts, without requiring culture in the laboratory, and can easily be delivered in suitable biomaterials; growth factors added to biomaterials may facilitate the differentiation of cells from the bone marrow.

Cartilage regeneration research should be accompanied by the development of new arthroscopic techniques to facilitate the fixation of the scaffold in the lesion, with the design of new instruments to allow repair in cases of patella and tibial plateau lesions. We should also not forget the need to establish the most effective physiotherapy protocols.

Conflict of interest

None declared.

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