Autologous Chondrocyte Implantation

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Primitivo Gómez-Cardero, E. Carlos Rodríguez-Merchán and Ángel Martínez-Lloreda

8.1 Introduction

Hyaline cartilage is a very important structure that plays a key role in optimal joint function. Lesions that result in the deterioration of the mechanical properties of hyaline cartilage may damage the joint and restrict its function. Although these are extremely common lesions, their natural history is not well understood and it is difficult to determine whether they will result in joint damage or remain stable causing no symptoms.

The study and treatment of such lesions is a challenge that must be faced by any orthopaedic surgeon determined to prevent their progression. However, success in this endeavour will require the support of other professionals such as molecular biologists and tissue engineers to support the development of new therapeutic alternatives that may provide an appropriate solution to the problem.

The prevalence of cartilage lesions in the knee joint ranges from 20 to 60 % [1, 2]. Of these approximately 7–11 % are grade III/IV lesions according to the International Cartilage Repair Society (ICRS) classification [3, 4]. These lesions are amenable to and would certainly benefit from early treatment so as to arrest the progression of degenerative changes in the joint.

Although the natural history of these lesions is poorly understood and difficult to predict, there is no doubt that chondral defects are apt to provoke high rates of morbidity in patients afflicted by them and may even cause osteoarthritis [4].

The quality of life of patients with focal articular cartilage lesions is often severely compromised. Indeed, the deterioration caused by these injuries can at times be comparable to the damage observed in patients with an anterior cruciate ligament tear or in those requiring knee arthroplasty or an osteotomy following severe osteoarthritis [5].

Articular cartilage lesions can add up to a substantial cost to society as these patients often present with significant levels of disability, which results in high levels of absenteeism, a lower quality of life and, eventually, knee replacement procedures. Therefore is would seem desirable to design an approach to these types of lesions that is as accurate and effective as possible in order to curb their progression and prevent the morbidity they have been shown to cause.

P. Gómez-Cardero (✉)
Department of Orthopaedic Surgery, "La Paz" University Hospital-IdiPaz, Paseo de la Castellana 261, 28046 Madrid, Spain
e-mail: gcarderop@hotmail.com

E. C. Rodríguez-Merchán
Department of Orthopaedic Surgery, "La Paz" University Hospital-IdiPaz, Paseo de la Castellana 261, 28046 Madrid, Spain
e-mail: ecrmerchan@gmx.es

E. C. Rodríguez-Merchán
School of Medicine, "Autónoma" University, Madrid, Spain

Á. Martínez-Lloreda
Orthopaedic Surgeon, Department of Orthopaedic Surgery, "La Paz" University Hospital-IdiPaz, Paseo de la Castellana 261, 28046 Madrid, Spain
e-mail: angelmlloreda@gmail.com

E. C. Rodríguez-Merchán (ed.), *Articular Cartilage Defects of the Knee*,
DOI: 10.1007/978-88-470-2727-5_8, © Springer-Verlag Italia 2012

A wide range of procedures have been developed in an attempt to resolve the problem. These can be classified into cartilage repair techniques (bone marrow stimulation through perforations or microfractures) and cartilage restoration techniques (osteochondral autografts and allografts and autologous chondrocyte implantation (ACI)).

ACI is the only technique capable of producing cartilage that is similar to native hyaline cartilage without the limitations of other restoration techniques. In this respect, osteochondral autografts and mosaicplasty are associated with donor site morbidity. In addition, the limited amount of tissue obtained makes these techniques unfeasible for large chondral lesions. Osteochondral allograft techniques, for their part, combine the difficulties inherent in harvesting fresh allografts with the potential risk of disease transmission.

Lindahl demonstrated that as far as patients with chondral lesions are concerned, ACI had a much higher cost-saving effect in terms of disability and absenteeism than any other technique [6].

The purpose of the present chapter is to discuss the state of the art as regards lesions of the articular cartilage and their treatment by means of ACI as well as the clinical and histological implications of this new therapy and its future prospects.

8.2 Structure and Function of Articular Cartilage

Articular cartilage is a kind of connective tissue endowed with a specialised structure conceived to provide joints with low-friction bearing surfaces capable of withstanding high loads, withstanding wear and allowing smooth joint motion. Their structure, however, has certain limitations in connection with its reparative capacity. Indeed, articular cartilage is devoid of vascularity and innervation, which means that it cannot resort to any self-healing mechanism when it suffers some kind of aggression that alters its mechanical structure [1–5, 7, 8].

As articular cartilage has no vascularity or innervation, nutrients and oxygen are supplied through passive diffusion from the synovial fluid. Nociception arises from the activation of the nerve endings of the synovium, the joint capsule, the muscles and the subchondral bone.

Hunter was the first author who, in 1743, made a description of chondral lesions in the knee and noted their poor healing potential [9]. The most common symptoms of full-thickness (grades III/IV) cartilage lesions in the knee are pain, inflammation, mechanical and functional alterations and, eventually, degenerative changes (osteoarthritis).

Hyaline cartilage is characterised by a high degree of specialisation and by a series of mechanical properties that make it possible for the joint to function appropriately, with a very low friction coefficient and high load resistance [10]. Its histological structure comprises the extracellular matrix and one single cell type: the chondrocyte.

Chondrocytes are the chief components of the extracellular matrix. As these cells have low replicative potential, their reparative response in the face of an attack is rather limited.

The extracellular matrix is a tridimensional structure that supports the chondrocytes and plays a decisive role in the chemical and physical processes that make it possible for the composition and the structure of hyaline cartilage to remain unchanged in the face of an attack. The matrix comprises 60–80 % water, glucose and salts, as well as chondro specific collagen (types II, VI, IX, X and XI), proteoglycans and other binding proteins and fibronectin [6].

Collagen fibres form a dense and interwoven network that contributes resistance to the tissue; 80 % is type II collagen. These collagen networks entrap the proteoglycans, constituted by monomers bound to chains of glyco polysaccharides, the most important of which are hyaluronate, chondroitin sulphate and keratan sulphate. Proteoglycans are capable of retaining water, thus keeping hyaline cartilage well hydrated so that it can preserve its biomechanical properties.

A thin film of synovial fluid covers the articular cartilage and diffuses into it carrying with it a supply of nutrients and water, decreasing the friction between the bearing surfaces during movement.

Articular cartilage comprises several layers, which are markedly different from one another in terms of cell shape and the density, biocellular

activity, composition and characteristics of the extracellular matrix and organisation of the collagen fibres. The superficial layer, which facilitates the sliding of the articular surfaces, boasts the greatest cell density and to the largest amounts of type II collagen. The intermediate and radial layers, responsible for cushioning the underlying bone, contain large amounts of type IX and X collagen and other proteins (cartilage oligomeric matrix protein and cartilage intermediate layer protein) [11, 12]. These differences are essential for optimal cartilage function. Therefore any attempt at cartilage repair must create a tissue structure that closely resembles the native tissue [12, 13].

8.3 Pathophysiology

Articular cartilage possesses a structure that allows it to withstand the loads and repetitive stresses it is normally exposed to. The superficial loads borne by cartilage vary depending on the type of physical activity performed. Walking, for example, has been shown to generate forces of up to 2.3 times body weight; running produces forces of 3.5 times body weight; tennis playing, 6 times body weight, skiing, 8 times body weight and playing squash 13 times body weight.

Nevertheless, cartilage is vulnerable to certain high-energy or repetitive forces that can result in alterations in its histological structure and cause a lesion that disrupts the cartilage's biomechanical properties. These forces are most commonly of traumatic origin, but they may also be produced by metabolic diseases (hyper- or hypoparathyroidism), alterations in the lipid metabolism, inflammatory processes (rheumatoid arthritis, haemophilic arthropathy, etc.), infectious processes or the existence of some genetic component.

Cartilage injury can be classified using the Outerbridge or ICRS scales [2, 14–16]:

1. The Outerbridge and ICRS scales classify chondral lesions into four stages (Fig. 8.1):
   ICRS grade 0: normal cartilage
   ICRS grade 1: softening and inflammation of cartilage

Ia: slight softening and mild fibrillation

Ib: superficial laceration and fissures

   ICRS grade 2: superficial fissures that involve less than 50 % of the cartilage thickness or smaller than 1.5 cm in diameter
   ICRS grade 3: defects involving more than 50 % of the cartilage thickness, or larger than 1.5 cm in diameter

IIIa: defects that do not involve the calcified layer

IIIb: defects involving the calcified layer

IIIc: defects that extend down to but not through the subchondral bone

   ICRS grade 4: absence of cartilage with exposure of subchondral bone

2. Osteochondral lesions, osteochondral fractures and osteochondritis dissecans.

The ICRS and Outerbridge scales also provide a correlation between the different grades of lesion severity and MRI (Magnetic Resonance Imaging) images, although it must be said that MRI tends to underestimate the extent and the depth of the lesion [2, 16, 17].

When the articular cartilage is damaged, an increase is observed in cell apoptosis, which results in an imbalance in the structure of the extracellular matrix and a subsequent disorganisation in the collagen ultrastructure and a decrease in proteoglycan content. All of these changes lead to an increase in patency and a loss of resistance [11].

A process is started in the damaged areas whereby an attempt is made to repair the injured cartilage, with chondrocytes synthesising extracellular matrix. Nonetheless, given the chondrocytes' low replicative capacity, the tissue thus formed bears significant quantitative and qualitative differences with hyaline cartilage. Indeed, the repair tissue is fibrocartilaginous, dense and made up predominantly of type I collagen.

With the passing of time, the ageing of the articular cartilage is accompanied by a loss of chondrocytes and a decreased stimulus responsiveness. The matrix also ages, with a gradual destruction of the interconnections in the collagen network and a loss of proteoglycans. Once it sets in, cartilage deterioration is irreversible, resulting in the deterioration of the joint through the release of a series of cytokines such as interleukin 1 (IL-1), tumor necrosis factor alpha

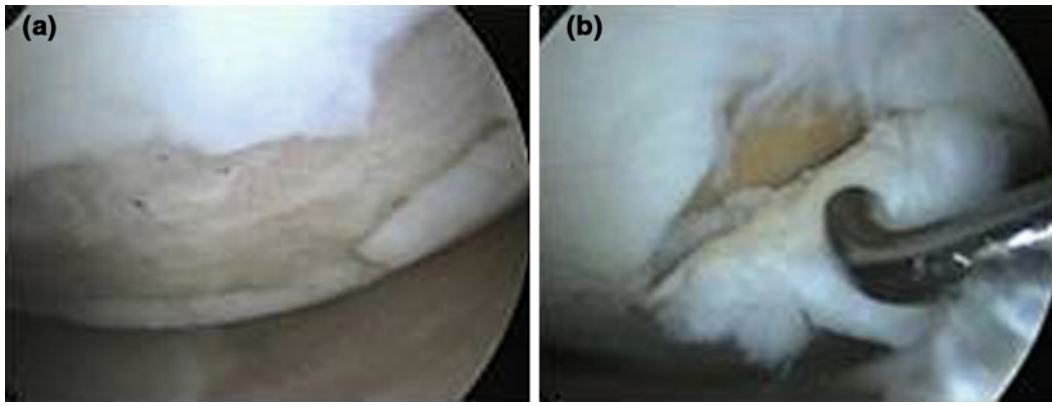

Fig. 8.1 ICRS (International Cartilage Repair Society) grade III (**a**) and IV (**b**) chondral lesions

(TFN-α) and enzymes that inhibit the synthesis of type II collagen and proteoglycans, leading to the appearance of osteoarthritis.

8.4 Diagnosis

When deciding what treatment is to be administered, an accurate diagnosis of the lesion is mandatory to establish an appropriate therapeutic algorithm. A detailed anamnesis is essential to gain insight into a series of variables that are key to the decision-making process.

1. *Patient-related variables*: patient age, body mass index, occupational or sports-related activity, history of the lesion, symptoms, the extent to which the patient is aware of the potential need of surgery as well as his/her expectations about the outcome of the procedure.
   The typical symptoms of a chondral lesion are typically mechanical: pain and inflammation on weight-bearing and intensification of symptoms on walking or running.
   Chronological patient age is not an absolute contraindication. It is on the other hand their physiological age that determines the most suitable type of treatment. There are studies that state that physiological age is a significant predictive factor for outcome in patients over 35 years [18, 19]. Better results are also obtained in patients with an active lifestyle [18].
   In terms of the time elapsed from the first symptoms, results have been shown to be best when less than 3 years have gone by since the onset of symptoms [19–21].
2. *Defect-related variables*: Location, size, depth and geometry of the defect; condition of the subchondral bone and the surrounding cartilage. The condition of the opposing surface, often underestimated (kissing lesions).
   Although the location of the lesion has not been shown to influence final outcome [21], its extent does seem to play an important role. In this respect, lesions <4 cm^2 have obtained better results when treated with either microfractures or ACI, but results in lesions >4 cm^2 were better only when treated with ACI [18, 22].

Physical examination should determine the presence of any concomitant alterations in the joint: malalignment, insufficiency or disruption of the cruciate ligaments and meniscal lesions.

The final diagnosis will be provided by imaging techniques. Weight-bearing and whole limb radiographs should be the first-line images as they offer an accurate representation of the mechanical and anatomic axes.

MRI is the safest and most reliable non-invasive method to diagnose chondral and osteochondral lesions, with a sensitivity in excess of 99 % [17]. MRI contributes enough information to carry out an appropriate preoperative plan, obviating the need to conduct an arthroscopic analysis of the lesion. However, it must be remembered that MRI often underestimates the extent and the depth of the lesion [2, 16, 17].

8.5 Treatment

Treatment of articular cartilage lesions is aimed at improving joint function, preventing the progression of joint damage and relieving or suppressing pain so that patients can return to their previous activity levels. If the repair provided is permanent, joint deterioration and the subsequent development of osteoarthritis will be staved off, which is beneficial both to reduce patient suffering and from a socioeconomic point of view.

The natural history of chondral and osteochondral lesions is still unknown and difficult to predict. Nonetheless, clinical experience suggests that when left untreated, these lesions do not heal spontaneously and may progress to joint degeneration [4, 23, 24].

Decision-making as regards the treatment of cartilage lesions in the knee joint must be tailored to each patient and to each individual type of injury [25]. In the first place, any concomitant disorder must be analysed: malalignment, any ligament tear leading to instability and meniscal lesions. These alterations must be treated simultaneously by means of femoral or tibial osteotomies, or tibial tuberosity transfers to correct varus or valgus deformities or a patello-femoral pathology. On other occasions, ligament reconstructions or even meniscal transplants may be necessary [26].

The possibility of primary repair will be considered in presence of an acute or subacute osteochondral lesion with an unstable fragment that is amenable to fixation. These lesions are typically larger than 1 cm^2 and are usually located in a weight-bearing area of the femoral condyles [25].

In patients where primary repair is not an option, the surgeon may resort to different treatment strategies:

1. Palliative techniques
2. Reparative techniques
3. Restorative techniques

Of these techniques, those seeking to produce hyaline-like cartilage are those that have offered the best long-term results according to the literature.

8.6 Autologous Chondrocyte Implantation (ACI)

The emergence of new technologies such as tissue engineering and gene therapy has made it possible to develop therapies which, in the last few decades, have produced highly promising results in the repair of articular cartilage tissue.

Tissue engineering seeks to find an optimal way to repair damaged tissue through the implantation of cells, supporting scaffolds and biologically active molecules or genes [27, 28]. It is based on the use of cartilage-producing cells as supporting structures that may stimulate cartilage repair and regeneration inducing the expression of molecules that may allow cell proliferation and differentiation [28].

ACI applies these tissue engineering techniques with a view to obtaining enough tissue to restore the joint surface, providing it with a histological structure and a mechanical response as similar as possible to those of the native cartilage.

This type of treatment is indicated in:

1. Symptomatic ICRS grade III and IV lesions in the femoral condyle, the trochlea or the patella
2. Lesions between 1 and 10 cm^2
3. Lesions where other techniques such as mosaicplasty and microfracture have failed
4. Motivated and active patients between 15 and 55 years of age

ACI is contraindicated in rheumatoid arthropathies such as rheumatoid arthritis, psoriatic arthropathy and infectious arthropathy. Kissing lesions are also considered a contraindication, although ACI has obtained promising results in patellofemoral lesions [29, 30].

In 1994, Brittberg and Peterson pioneered the use of ACI in lesions of the articular cartilage of the knee [31]. They obtained chondrocytes from their patients, which they cultured and expanded in vitro to subsequently implant them under a periosteal flap. Since then, over 12,000 patients have benefitted from the technique.

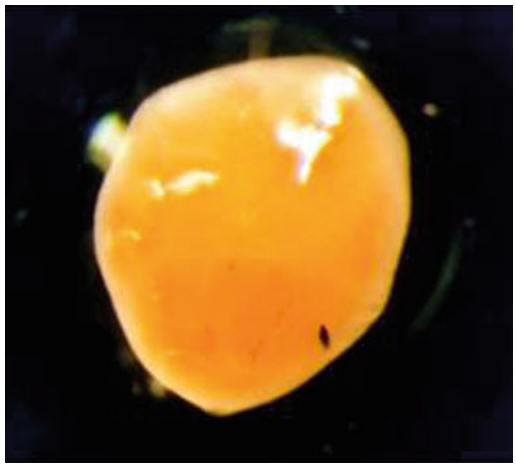

Fig. 8.2 Three-dimensional microsphere-shaped structure (pre-aggregated human articular chondrocytes)

Multiple trials have been conducted both in vivo and in vitro to gain a better understanding of the characteristics, function and behaviour of chondrocytes so as to improve the ACI technique and standardise the analysis of results.

ACI is a technique that has significantly evolved since its inception. At first, the culture and expansion of chondrocytes gave rise to a single-layer structure that was then implanted into the defect and covered with a periosteal layer. One of the drawbacks of this is that two-dimensional single-layer structures promote cell dedifferentiation and bring about changes in cell behaviour and morphology as well as a decrease in the production of articular cartilage-specific proteins [32].

For this reason, an attempt was made to create three-dimensional cell cultures as these have been seen to preserve their chondrogenic potential and to have lower dedifferentiation rates than single-layer cultures [33, 34] (Fig. 8.2).

The first generation of ACI, called ACI-P, involved the use of a periosteal patch. The chondrocytes were harvested, cultured and expanded as a single layer with the assistance of growth factors to be then implanted into the defect. These chondrocytes were subsequently covered by a periosteal patch, which functioned as a seal isolating the chondrocytes so as to prevent them from leaking from the graft site.

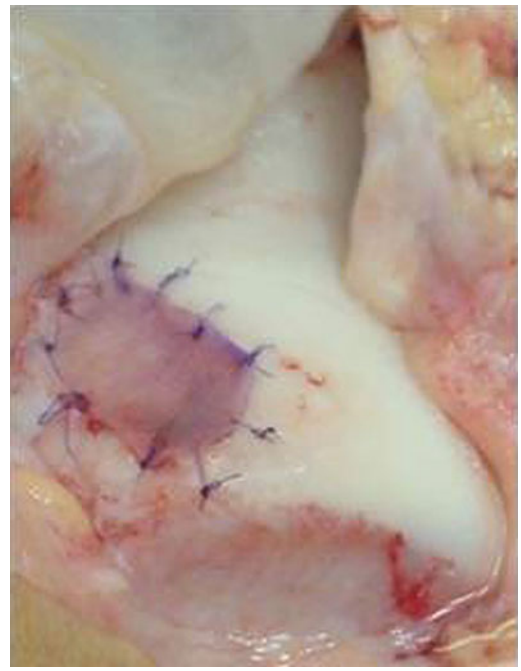

Fig. 8.3 First generation autologous chondrocyte implantation (ACI): chondrocytes had to be covered by a periosteal patch

The next generation was called ACI-C and used scaffolds made of collagen of animal origin. This technique had a series of drawbacks, including morbidity of the periosteal graft donor site and the difficulty of the surgical technique which involved an arthrotomy as well as the suturing of the scaffold to the borders of the defect. The technique was associated with a series of complications such as arthrofibrosis (up to 15 %), periosteal membrane hypertrophy (up to 25 %) and graft delamination. Such complications require reoperation in up to 50 % of cases [18, 35–38].

All these problems led to the development of the next generation of ACI: Matrix Induced Autologous Chondrocyte Implantation (MACI). MACI scaffolds are three-dimensional biological structures of variable composition, structure and porosity levels. The most common scaffolds are those made of collagen, demineralised bone matrix or hyaluronic acid. The chondrocytes are seeded into the scaffold, which can be adhered directly to the base of a prepared chondral defect without a periosteal cover (Fig. 8.3).

MACI offers a series of advantages over the ACI procedure: it may be performed arthroscopically, it requires less operating room time and rehabilitation is usually faster. Several studies have obtained good results using different types of scaffolds as chondrocyte supporting structures [39–45].

Nevertheless, it must be stated that the MACI technique is not devoid of complications such as subchondral edema, synovitis and foreign body reaction. All of these complications are associated to the exogenous matrix used as a scaffold.

Several studies in the literature compare ACI with other existing techniques. Knutsen [18] compared ACI-P with microfractures in a series of 80 patients and observed that in lesions larger than 4 cm^2 the microfracture technique yielded poorer results. In a series of 118 patients, Saris [46] concluded that the repair tissue that developed within 1 year post-op with ACI was structurally better than that obtained with the microfracture technique, although his clinical results were similar.

Dozin [47] compared the use of an osteochondral autograft with ACI and concluded that both techniques afford similar clinical results. In a series of 100 patients, Bentley [48] found that both clinical and histological results were significantly better with ACI than with mosaicplasty. Bhosale [49] observed that 81 % of patients who underwent an ACI procedure improved within the first 15 months and this improvement was still present at 8 years.

More recently better-designed and more scientifically rigorous studies have been published which show a trend toward better outcomes with ACI as compared with other techniques [18–22, 27, 39, 46, 47, 50, 51]. Nevertheless, results are still extremely variable, which means that it is too early to state that ACI is more effective than any of the other better established techniques supported by large bodies of scientific evidence. For this reason, ACI is still considered a second-line therapy for lesions of the articular cartilage [21, 52, 53].

There is at present a wide range of tissue engineering systems and techniques aimed at obtaining cells which, when implanted in vivo into a chondral lesion, may effectively repair it. However, multiple factors that critically influence the development and formation of these cell structures remain to be addressed [54].

8.7 Two Surgeries

The ACI technique requires two surgical stages. In the first, a cartilage biopsy is taken from a non weight-bearing area and, in the second, the new chondrocytes are implanted. Several investigators are exploring the possibility of performing the procedure in one single stage but these trials are still in progress and no conclusions have been reached as yet. The fundamental idea behind these investigations is that the articular graft harvested may be cut into pieces and placed onto a scaffold which is implanted into the chondral defect in the same surgical act [55, 56].

8.8 Cell Dedifferentiation

When chondrocytes are cultured and expanded in a single-layer, they undergo phenotypic dedifferentiation, which results in changes in their morphology and the production of extracellular matrix [32]. Studies show that this problem could be overcome by producing three-dimensional structures where chondrocytes can redifferentiate [34]. Use of growth factors also favours cell dedifferentiation [57, 58].

Nevertheless, it has been shown that cell dedifferentiation is not such a major impediment to the production of viable chondrocytes as dedifferentiated cells preserve their ability to redifferentiate.

8.9 Scaffolds: Use and Characteristics

Multiple structures of different architectures and mechanical properties have been used as scaffolds. In the main, they are three-dimensional polymers that contain proteins and natural polysaccharides or synthetic polymers such as polyglycolic acid, polylactic acid or hyaluronic acid. One of the main problems about these structures is that they must be mechanically strong and

Fig. 8.4 Microsphere-shaped three-dimensional structures once cell culture and expansion have been completed

biodegradable at the same time. Furthermore, it is essential to create a homogeneous distribution of a sufficient number of cells over the scaffold's surface. In this respect, bioreactors have been used to deliver the chondrocyte suspension directly into the pores of the scaffold [59].

The ideal scaffold must be strong in order to protect the cells contained within it against mechanical forces. It should also be capable of adhering to the defect and be porous enough to allow local growth factors to penetrate the surface. It should also be biodegradable and eventually eliminated so as to prevent any foreign body reactions [28].

A technique has been developed that does not require the use of exogenous scaffolds. This is a highly advantageous breakthrough as it completely precludes potential foreign body reactions as well as problems related with resorption, hypertrophy or calcification. It is a technique that uses adult chondrocytes grown and expanded in the patient's own serum, without exogenous growth factors or antibiotics. In this manner, microsphere-shaped three-dimensional structures are created, with around 200,000 chondrocytes each, which are capable of adhering to the chondral defect without any other coverage or anchoring device [34] (Figs. 8.4 and 8.5).

8.10 Cell Variability

The quality of the sample obtained to start the whole process is one of the keys to success. Quality varies depending on the condition of the joint, the patient's age and is even different across individuals of the same age [60]. Although several specific chondrogenesis markers have been identified in an effort to standardise the results of cell cultures, attempts at developing a standardised tissue engineering method to obtain an articular cartilage graft have as yet been unsuccessful [54].

8.11 Chondrocyte Sourcing

The fact that chondrocytes are obtained from the patient's own joint is one of the main disadvantages of this technique, as the procedure may result in donor site morbidity. Although only a small biopsy is taken from a non weight-bearing area of the joint, some studies claim that this may increase the future risk of developing osteoarthritis [61, 62].

In order to overcome this problem, alternative chondrocyte sources have been proposed. Among of the most promising of these are pluripotent mesenchymal stem cells, which may be obtained from the bone marrow, abdominal fat or the knee and the synovium. These cells have a high proliferation capacity and a low dedifferentiation potential and, with appropriate growth factors and signals, they can induced to differentiate to cartilage cell lines.

Nonetheless, these new chondrocyte sources are not exempt from difficulties as the different steps required for cell division significantly decrease their chondrogenic potential. In addition, the use of growth factors leads to chondrocyte phenotypic instability with a loss of extracellular matrix secretion [28].

In order to overcome these problems, cultures of mesenchymal stem cells have been used together with adult chondrocytes since the latter express growth factors that may act on mesenchymal cells to induce chondrogenesis [63, 64].

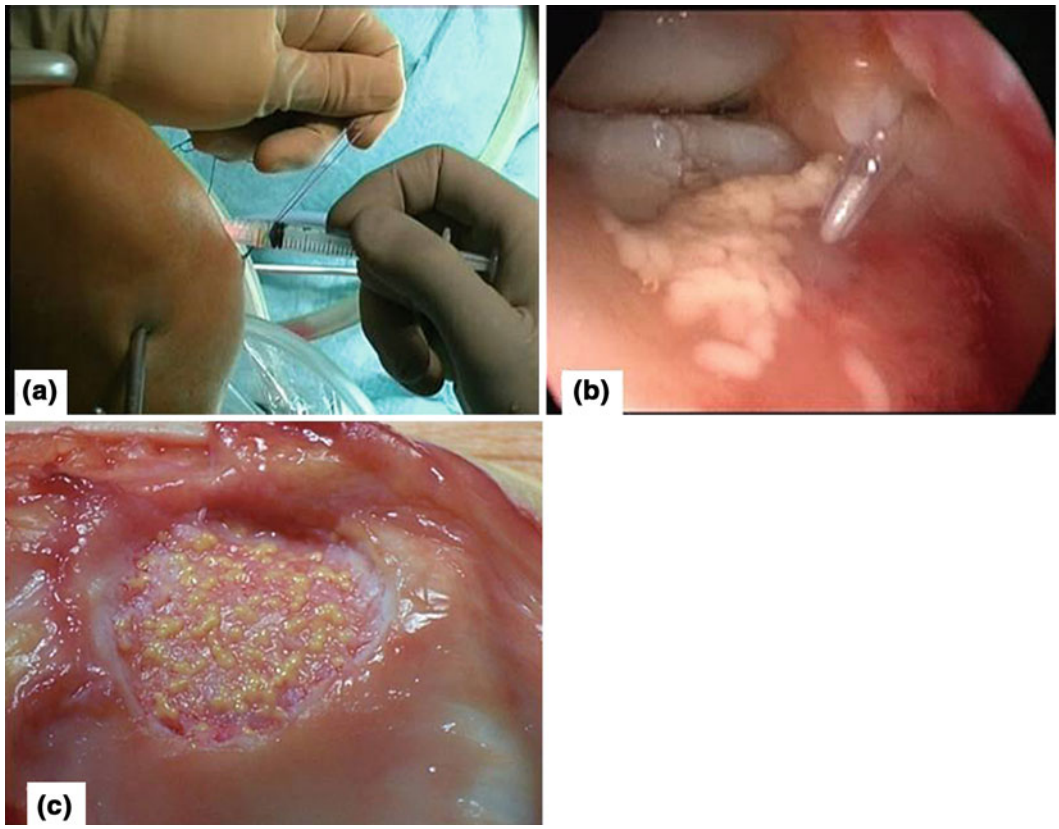

Fig. 8.5 Arthroscopic application of chondrospheres to the chondral defect (**a** and **b**). View of the defect with the chondrospheres in place (**c**)

When these cells differentiate to chondrocytes they also express markers such as type X collagen and MMP13, which are specific to chondrocyte hypertrophy and extracellular matrix calcification and vascularisation following transplantation [65, 66]. These alterations could result in phenotypic instability and a decrease in the long-term efficacy of chondral regeneration. Therefore, although multiple studies have successfully used these cell types both in animals and in humans [67–70], the efficacy of mesenchymal cell-based treatment remains to be conclusively demonstrated [54].

An alternative claimed to prevent phenotypic instability of mesenchymal cells is the use of mature chondrocytes from non-articular cartilage. Several authors have used nasal cartilage. These chondrocytes have a higher replication capacity than articular chondrocytes and, once expanded, maintain their ability to generate hyaline cartilage both in vitro and once transplanted in vivo [71–74]. It has also been argued that the mechanical properties and biochemical and histological characteristics of these cells are similar to those in the native hyaline cartilage, and they seem to be superior to those in the tissues obtained from articular chondrocytes [74].

It appears that tissue engineering is a technique that could be of great value in the study and—most importantly—in the repair of articular cartilage lesions. However, there still remain a few hurdles that need to be overcome in order for these therapies to be standardised and made widely available.

8.12 Conclusions

Articular cartilage lesions are a common pathology in the knee joint. Treatment of these lesions (microfractures, mosaicplasty, ACI, MACI) must be individualised taking into account variables such as location and extent of the lesion, the patient's activity level and economic cost. Well-designed multi-centre studies need to be conducted before determining what kind of treatment is the most effective. The literature seems to indicate that tissue engineering-based therapies are the only ones capable of forming tissue whose histologic and biomechanic characteristics are similar to those of hyaline cartilage. Nevertheless, none of these techniques are as yet standardised or reproducible, nor do they offer at the present time results that can be considered significantly superior to those of other existing therapies.

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