Chapter

Cartilage Tissue Engineering and Regeneration

Francisco Sousa Segundo, Marcelo Jorge Cavalcanti de Sá and Roberto Lopes de Souza

Abstract

Cartilaginous tissue is mainly present in the joints, and it consists predominantly of type II collagen and glycoproteins, which promote functions of supporting biomechanical forces generated during the ambulation. The cartilage has a very limited regenerating capacity, causing traumas or degenerative diseases in this region difficult to solve. The current treatments for regeneration of the articular cartilages may be conservative or surgical, but they are not very successful, since the damaged tissue is replaced by fibrous tissue or fibrocartilage, with predominantly type I collagen, which present inferior functions. Cellular therapies, biomaterials, and tissue engineering to assist the healing process have been showing great potential. For example, the in vitro chondrogenesis of mesenchymal stem cells (MSCs) is a technique that stimulates undifferentiated cells to transform into chondrocytes, creating a dense mass of aggregated MSCs and an environment with strong cell-cell interactions.

Keywords: biomaterials, cell therapy, joint, tissue repair, regenerative medicine

1. Introduction

Joint diseases such as osteoarthritis can cause important lesions in the articular cartilage; in humans, this pathology can affect a significant proportion of patients over 60 years of age, causing a great negative impact on their quality of life. This increased the search for an effective treatment the objective of several researches, often seeking the cooperation of several areas of knowledge; however the difficulty in repairing articular defects in an effective way is becoming a real challenge for medicine [1].

The cartilaginous tissue present in the joints is a highly organized and specialized tissue, presenting several fundamental mechanical proprieties for the maintenance of articular function [1].

The lesions caused in this tissue from trauma or degenerative diseases cause a gradual damage to the tissue, leading to joint pain and consequent impairment in its function, which are difficult to handle clinically [1].

Thus, cases of severe joint disease are usually treated surgically, either through osteotomies and the application of autologous subchondral grafts, reducing the progression of the cartilage lesion and promoting return to joint function, or, in more severe cases, complete articular replacement through prosthesis implantation [2].

Due to the fact that these techniques may present unsatisfactory clinical results, alternative treatments for repair of this type of injury are constant aims of research, mainly in the area of regenerative medicine and tissue engineering, which are commonly working together for the development of different therapies [2].

With the development of regenerative techniques, such as the use of biomaterials and implantation of autologous cells or tissue, promising results emerged; it was also noticed that the association of several technical modalities was indispensable for obtaining satisfactory clinical recoveries [3].

2. Cartilage proprieties

The articular cartilage is formed basically of hyaline cartilage, being rich in type II collagen fibers and glycoproteins; this tissue is present in the end of the long bones and sesamoids with synovial articulation, as well as in the physeal line, which divides the diaphysis and epiphysis and is responsible for part of the bone growth [4].

The healthy articular cartilage has a macroscopic aspect with shiny and whitish color and smooth surface, is always bathed by the synovial fluid which is produced by the synovial membranes, and is contained in the articular capsule. Its main function is the cartilage nutrition and articular lubrication, and its translucent and viscose consistency contributes to the sliding of the articular segments and reduction of the articular friction during the movement [5].

Histologically the articular cartilage is defined as a highly specialized tissue with cells called chondrocytes, which produce collagen fibers, distributed in rows, in the periphery of the tissue; these cells present in elliptical form with the largest axis parallel to the tissue surface, in the center of the cartilage, and have a rounded shape and can group together. Another component present in the cartilaginous tissue is the amorphous substance which is composed of macromolecules of glycosaminoglycans [6].

This tissue has little or no vascularization, and its nutritional supply comes almost exclusively from the synovial fluid. This makes it difficult for cell migration and proliferation in injured sites; due to this, treatments through microfractures, where a surgical perforation is performed in the cartilaginous lesion aiming to cause hemorrhage and clot formation from the subchondral bone, are widely employed. Despite being one of the most used techniques, clinical results are often unsatisfactory [7].

The cartilaginous tissue has almost no innervation, which favors its function of capturing and distributing mechanical forces applied to it during movement, without any painful reaction. Full-thickness lesions in the cartilage cause the subchondral bone tissue lying just below the cartilage to be exposed, and the friction generated during movement causes contact with the subchondral bone to cause a debilitating pain for joint function [8].

The impairment of the normal function of a joint can negatively influence several nearby structures, and the lack of adequate use of a limb with joint problems leads to atrophy of muscle groups, which in addition to having clinical impacts on patients' quality of life can still make it impossible for some patients to work [8].

2.1 Cartilage lesions

Initially it was believed that osteoarthritis (OA) occurred due to wear and tear of the articular surface; however, it is now understood that the development process of such pathology has much more complex mechanisms [9].

The changes observed in the joints affected with OA are usually directly related to the severity of the same, being observed formation of osteophytes, inflammation

of articular and periarticular components, bone deformity, and degeneration of the cartilaginous tissue, as shown in **Figure 1** [11].

Several causes can lead to impairment of the normal function of articular structures, including trauma and degenerative diseases, culminating mainly in mechanical instabilities, which promote cartilage surface abrasion and progressive degeneration [12]. This is due to the release of cellular communicators such as cytokines; interleukin types 1, 4, 9, and 13; and tumor necrosis factor alpha (TNF-a), as well as release of enzymes such as disintegrin, metalloproteinase thrombospondin-like motifs, and collagenases; all this activity is carried out by chondrocytes, osteoblast, and synoviocytes, as shown in **Figure 2** [14]. It is also believed that the innate immune system may participate in the progression of OA, mainly by the activation of the complement and alternative pathways [15].

After stimulation, the release of enzymes in the joint leads to degradation of the cartilaginous matrix, causing chondrocyte hypertrophy, which loses the ability to produce a new collagen matrix [16]. The proliferation of the subchondral bone exceeds the intersection between the bone and cartilage, causing the formation of osteophytes, subchondral cysts, and subchondral sclerosis; all of this process aims to compensate for a mechanical instability in the joint and redistribute the forces acting on it [17].

However, the continuous exposure of the tissue to mechanical stimuli leads to the release of vascular endothelial growth factors by chondrocytes, which promote intense neoangiogenesis and invasion of the joint tissues. Patients affected by angular deviations or instabilities that increase joint exposure to poor distribution of mechanic forces have a much more aggressive progression of OA, with subchondral bone damage associated with severe articular pain [18].

In these cases pain may be associated with the remodeling of the subchondral bone, which is widely innervated, and the inflammatory process of the joint structures may contribute to the aggravation of pain; in more chronic cases, the joint membrane may become fibrosed, which compromises the performance of its

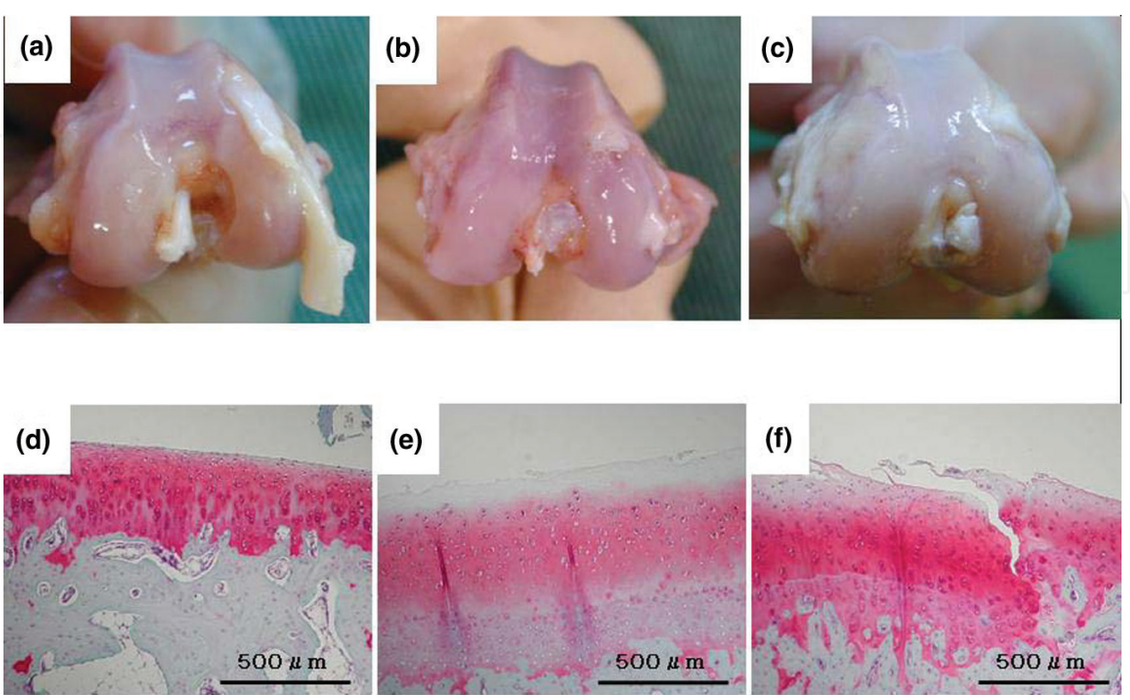

Figure 1.
Macroscopic findings of rabbit articular cartilage at 1 week (a), 4 weeks (b), and 8 weeks (c) after collagenase injection. Photomicrographs of rabbit articular cartilage at 1 week (d), 4 weeks (e), and 8 weeks (f) after collagenase injection are also shown (Safranin O staining; magnification × 4); taken with permission [10].

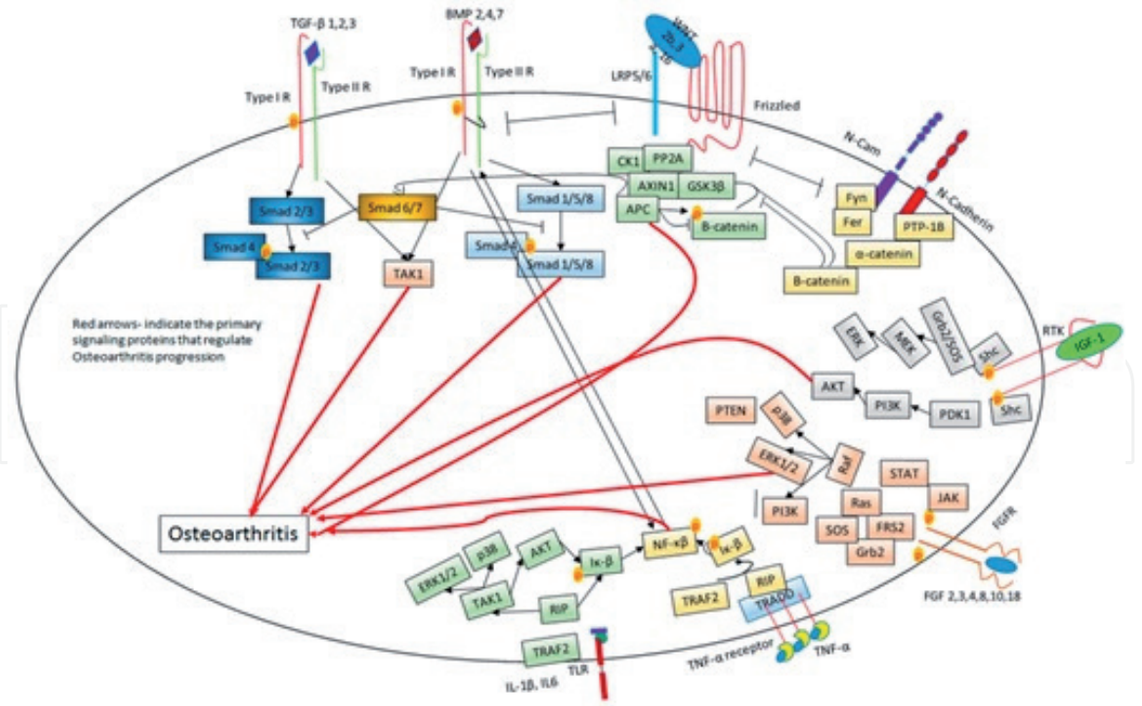

Figure 2.
Signaling cascades involved in osteoarthritis. Red arrows indicate the primary signaling protein that regulates OA progression. The black arrows signify the activation of the proteins. The bars indicate inhibition of the proteins; taken with permission [13].

normal functions, and analgesic treatment is of great importance and can be difficult if central or peripheral sensitization occurs [19].

The development of therapeutic modalities for the management of OA is a constant interest of researchers; commonly experimental animal models are used to investigate different treatment options, and among the most used animals are guinea pigs, rabbits, rats, dogs, sheep, and horses, studying OA in different joints, such as the temporomandibular, metacarpophalangeal, and the most frequently used knee joint [20].

The development of experimental models for OA is of fundamental importance for the understanding of the pathophysiology of the pathology, for example, through experimental models in rats as shown in **Figure 3**; it was possible to observe a much more complex relation between the histological morphology of the cartilaginous tissue and the pain phenotypes. It can contribute significantly in understanding the mechanisms of cellular development and interaction in diseased joints as well as in the targeting of patients' analgesic therapy [21].

2.2 Tissue engineering

Complications related to the surgical treatment of microfractures have caused the search for alternatives or associations to grow rapidly; one of the areas with promising results is tissue engineering, which cultivates cells or tissues in vitro, intended for implantation in injured sites [22]. Usually chondrocyte cells are collected from the patient, in joints that are not submitted to biomechanical loads; then the cells are cultured and multiplied in the laboratory, reaching amounts of 12 to 48 million cells, and then implanted in affected joints [23].

The main benefits of this technique are the low rate of rejection or complications due to foreign materials to the patient, since the base cells are collected by biopsy and are autologous to the patient and the noninvasive nature and since the

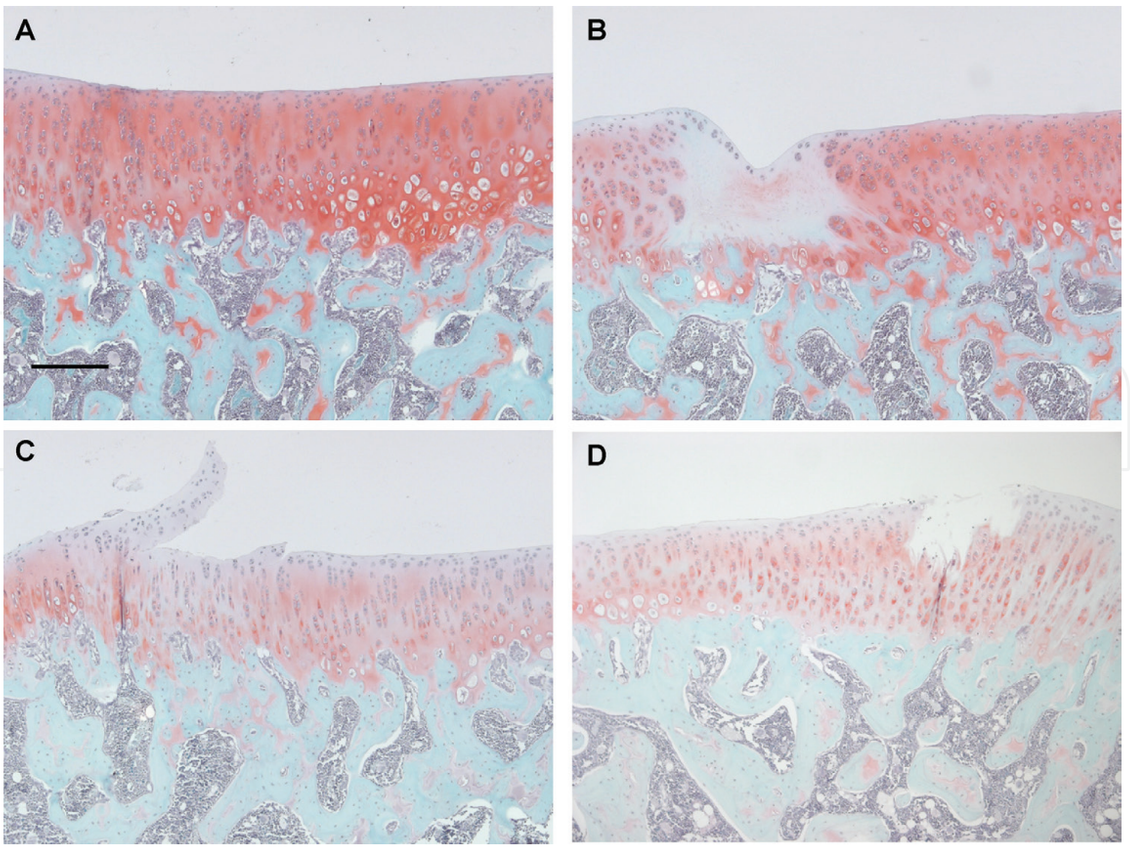

Figure 3.
Articular cartilage pathology 20 and 42 days after OA induction. Histological images of the tibial plateau (A–D). Joints were sectioned in a frontal plane and stained with Safranin O/fast green staining and corresponding consecutive sections stained with H&E. (A) Saline-treated control showing smooth cartilage and normal joint margin and chondrocyte morphology. (B–C) 1 mg and 0.1 mg glycolysis inhibitor monosodium iodoacetate-injected rats at day 20 and (D) 0.1 mg glycolysis inhibitor monosodium iodoacetate-injected rat at day 42 show degeneration of the cartilage. Proteoglycan loss (B–D) and chondrocyte cloning (B) are also present in the arthritic cartilage. Scale bars = 200 μm. Images are of knees with median cartilage surface integrity scores from each group; taken with permission [21].

collection and implantation are usually performed through arthroscopy, which promotes greater comfort and better recovery for the tissue [24].

The main negative points observed in this technique are the need for two interventions, a relatively long recovery time, between 6 and 12 months, which are necessary for the tissue neoformation and its maturation, and the intrinsic complexity of performing this procedure [25].

Clinical studies and follow-up of long-term cases have confirmed excellent clinical and functional results of patients submitted to autologous chondrocyte grafting in articular defects of up to 4 cm^2 [26]; positive results were also observed when associated with grafting technique with corrective osteotomy [27]; when compared with the microfracture techniques, the autologous chondrocyte grafting obtained superior results in joint defects of 3 cm^2 or greater, entertained, and when these two techniques were used in minor defects, no difference was observed between the treatments [28].

The autogenous chondrocyte graft appears to have limitations, especially in relation to patients with very large articular defects >15 cm^2 and with severe tissue lesions; in these patients the graft technique is associated with a low survival rate of the implanted tissue, and despite this, no clinical differences were observed with patients whose grafts were successful [26].

In humans the autologous chondrocyte implant seems to have a more favorable result when used in younger patients and with a joint disease in a period shorter than 12 months, with a rate of return of high impact sports of up to 96% of patients treated [29].

Despite promising long-term results, up to 10 years of follow-up, additional characterizations of the newly formed tissue after autologous chondrocyte implantation are required through imaging or arthroscopy [30].

An alternative to surgical implantation of tissue manufactured in vitro is the intra-articular application of pluripotent cells, such as allogeneic or autologous undifferentiated mesenchymal cells; these are derived from bone marrow, adipose tissue, or the umbilical cord [31]. This technique has been used mainly for the purpose of reducing joint pain and reducing the progression of tissue degeneration; between 8 and 9 million autologous cells are administered per patient or 40 million allogeneic cells per patient; administrations are performed in the affected joint and promote stimulation of chondrogenesis [32].

Despite reports of positive effects, the application of undifferentiated mesenchymal cells to OA treatment is still controversial, and the mechanisms of action of the use of this type of cells in a diseased cartilage are still not fully elucidated, suggesting both direct effect on the recovery of tissue through cell differentiation and indirect effect through the release of inducing factors and tissue growth, the two actions being associated with beneficial effects on the joint [33].

2.3 Biomaterials

The advances in material engineering in association with medicine have enabled new technologies to be developed to help treat various pathologies that were real challenges. Among the technologies developed, the manufacture of materials with various biological properties that could be implanted in different tissues was an event that brought excellent results and opened up a wide spectrum of possibilities [34].

Biomaterials must possess certain characteristics to optimize their benefits, for their use in the repair of cartilaginous tissue; besides being biocompatible and providing cellular adhesion and proliferation, they must be bioactive, biomimetic, biodegradable, and bioresponsive. These characteristics added to an adequate three-dimensional arrangement favor the environmental stimulus for the production of desirable cells, such as chondrocytes [35].

Among the materials that can be used in the manufacture of biomaterials are polymers which may be either natural or synthetic. Natural polymers have a better interaction with the implanted site, providing a more natural environment for cellular development, supporting and guiding their differentiation between several stages; however, one of its negative points is the low mechanical capacity when compared with other biomaterials [36].

Synthetic polymers can have their mechanical characteristics of controlled strength, stiffness, and degradation rate, making them quite versatile; since their biological characteristics are not desirable due to their hydrophobic properties, it is often necessary to add other materials that increase your cellular interaction [37].

A collagen-based implant, developed with three layers, made with the combination of equine collagen, magnesium, and hydroxyapatite, showed a good result in patients with large joint defects; however, the number of cases was small, suggesting that more studies are required [38]. Polyglactin-based implants associated with platelet-rich plasma and hyaluronic acid have shown promising results in the treatment of joint injuries; however, larger studies are needed [39].

In addition to the regenerative properties of biomaterials, these can still be used as controlled drug delivery systems, among which the most studied are microparticle implants manufactured from poly(lactic acid), poly(lactic-co-glycolic acid), and polycaprolactone—these synthetic polymers are made up of particles measuring above 1 μm [40].

Studies using poly(lactic acid)-based microspheres as a controlled drug delivery system were able to obtain a drug release rate of 20 to 62% in 3 months when applied in vivo; the biomaterial has shown bioactivity 2 months after intra-articular application in rats, showing potential for pain control in patients with OA [41].

The same biodegradable synthetic polymers can be used to manufacture nanoparticles, which have a size smaller than 1 um, which can also carry drugs with analgesic, anti-inflammatory, or other biomaterials with regenerative properties [42].

When compared to the microparticles, the nanoparticles have a shorter action time, being eliminated in a matter of days, as observed in the study applying microparticles and nanoparticles based on chitosan, in rats with OA [43].

Another class of biomaterials is ceramics; these are widely used in the repair of bone defects, due to their excellent properties of osteoconductivity and osteoinductivity; although they have lower mechanical characteristics than other materials, their structure can be manipulated and associated with other products [44]. By associating ceramics with type 2 bone morphogenetic protein, stimulation was observed for subchondral bone growth, as well as for the cartilaginous tissue itself [45].

2.4 Conservative therapies

The drug treatment for patients with OA has as main target the control of joint pain, which is the main reason for the patients to seek medical attention [46]. Often clinical pain arises before radiographic changes, and its etiology is not fully understood; however, the inflammatory signs produced by chondrocytes seem to play an important role for joint pain [47].

The main medications used to control joint pain are nonsteroidal anti-inflammatory drugs, opioids, or the combination of both; supplementation of vitamins, hormones, and chondroprotective medications is also widely employed [48]. Meloxicam and tramadol are drugs commonly used to control pain; however, studies have shown that long-term use of these drugs can bring several side effects, including headache, nausea, diarrhea, and urinary tract infection [49, 50].

Intra-articular administration of viscous substances can also be performed; basically all products are based on hyaluronic acid, which is produced by fibroblasts and has the function of lubricating the joint [51]. Its application showed superior results to the use of placebo in the return to joint function and reduction of pain in patients with OA [52].

Rehabilitation is a fundamental element in the recovery of joint function; this modality can be used alone or in association with other therapies. The main objective is that the cartilaginous tissue adapts through exercises that promote a regular mechanical stimulus in the joint; this process is quite slow, since this tissue can take up to 2 years to reach 75% of adaptation, contrasting muscle tissue which reaches a total adaptation at 35 weeks [1].

Even with the development of several techniques and technologies for recovering patients with joint disease, physical exercise is always present in therapeutic protocols, showing that the continuous passive movement or range of motion exercises practiced from the first day after surgical interventions is fundamental for obtaining better results [53].

Studies show that the early use of controlled exercises in OA patients promotes results superior to immobilization, contributing to decrease edema, early return to physical activity, restore range of motion, articular stability, and improve patient satisfaction with the therapeutic outcome [54].

2.5 Surgical techniques

Surgical interventions may be options for the treatment of OA, in cases in which the patient has clinical signs, and the osteotomy technique and autologous

osteochondral implantation (ACI) may provide a return to joint function and minimize the progression of cartilaginous degeneration and may be associated to other therapeutical modalities such as implantation of biomaterials or tissues produced by engineering; in more chronic cases, the most commonly used surgical technique is the total replacement of the joint by prostheses; however, the long-term results of these interventions do not present acceptable clinical solutions [55].

The microfracture technique aims to perforate the subchondral bone and stimulate the migration of pluripotent cells from the bone marrow to the injured site in the cartilaginous tissue; although it is considered a gold standard technique, generally this procedure produces a fibrocartilaginous tissue, which has inferior biomechanical properties when compared to the cartilaginous tissue [56].

The fragility of the fibrocartilaginous tissue produced by the microfracture process causes this procedure to promote a limited recovery of the tissue, and the neoformed tissue usually degenerates in 18 to 24 months after the procedure due to its biomechanical characteristics [57], which compromises the long-term positive results. Initially, a decrease in lesion progression is observed up to 5 years, after which time treatment failure is expected [58].

Follow-up after treatment was of great importance, as was shown in studies comparing microfracture and autologous graft of chondrocytes with a 5-year follow-up; no significant clinical difference was observed between the groups, and despite that, the samples collected through biopsy revealed that all cases of failure after intervention and the obtained tissue had inferior quality to hyaline cartilage [59].

The osteochondral autograft transplantation (OATS) consists of the collection and implantation of an autogenous or allogeneic fragment of the bone and hyaline cartilage for repair of the defect in a joint affected by OA; this technique presents better results when used for the correction of minor defects; among its negative points, one has the morbidity of the donor caused by the collection of the material for implantation [60].

Approximately 60% of patients with OA will at some point require total joint replacement or other salvage procedures such as arthrodesis and total joint replacement to remove severely injured joint segments by prostheses; patients undergoing arthrodesis have joint surface completely removed, and the implant segments are stabilized with implants for fusion to occur [61].

In spite of the invasiveness of these procedures, patients generally adapt well to the intervention, with significant pain remission and limb function returned; however, these data seem to be related to short- to medium-term follow-up, and their long-term efficacy is questioned [62].

3. Conclusions

The OA represents a complex and recurring problem in medicine, causing pain and debilitation to patients, and its effective treatment is a real challenge.

Several therapies can be employed; however, their efficiency is variable, and it is suggested that the association of more than one therapeutic modality is the best way for a better recovery.

Conflict of interest

The authors declare no conflict of interests.

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