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Updates in biological therapies for knee injuries: full thickness cartilage defect

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Abstract Full thickness cartilage defect might occur at different ages, but a focal defect is a major concern in the knee of young athletes. It causes impairment and does not heal by itself. Several techniques were described to treat symptomatic full thickness cartilage defect. Recently, several advances were described on the known techniques of microfracture, osteochondral allograft, cell therapy, and others. This article brings an update of current literature on these well-described techniques for full thickness cartilage defect.

Keywords Full thickness cartilage defect · Knee injuries · Microfracture · Osteochondral defect · Osteochondral allograft · Autologous chondrocyte implantation · Cartilage repair · Cell based therapy

Introduction

The articular cartilage is a highly specialized connective tissue. Therefore, it has the characteristics of being aneural, avascular, alymphatic and somewhat hypocellular, but with specific biomechanical features [1]. The cartilaginous tissue has a great amount of dense connective tissue, composed of cells, water and matrix. Its main functions are the protection of the subchondral bone, absorption of impacts and the connection of the bone articular structures. The primary components are the chondrocytes surrounded by extracellular matrix (ECM). The ECM is composed mostly of collagen type II fibers, proteoglycans and water [2].

Chondral injury has several clinical presentations and might occur at different ages [3]. Currently, due to changes in lifestyle of the world population and the growing number of sports participation, a gradual increase of injuries related to this structure has been observed and as a consequence, the increasing number of studies to understand and propose treatments [4].

The cartilage defects, particularly full thickness defects, have low or no capacity for regeneration due to its characteristics [5]. We can define the cartilage tissue as a structure of great importance for the movement and joint functioning however, it is extremely susceptible to irreparable damage.

The natural history of chondral injury as it affects the whole thickness has evolved into osteoarthritis and its clinical consequences, notably edema, pain and functional limitation. The intensity of symptoms associated with the degree of articular symptoms weakness of the patient may lead to the need for surgical replacement of the articular surface using knee arthroplasty [6, 7].

Currently, there are available enshrined methods of treatment, such as osteochondral allografts, microfracture, mosaicplasty, allograft and autologous chondrocyte implantation, depending on the characteristics of injury. Still in the therapeutic plan, several ongoing studies try to demonstrate the effectiveness of new therapies, such as platelet-rich plasma and mesenchymal stem cells, among others [8].

The purpose of this study is to discuss the existing biological treatments for chondral injuries, considering its most recent updates.

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Microfracture

Microfracture is one of the most reliable and used techniques for treatment of chondral lesion of the knee [9, 10••]. In 1999, Steadman [11] described a microfracture treatment where the cartilage defect is filled with progenitor cells derived from the bone marrow of the own patient. Multiple perforations of the subchondral bone are performed, promoting bleeding and migration of mesenchymal cells to cover the defect [11, 12].

Microfracture is the treatment of choice for small lesions (less than 2.5 cm^2). It improves symptoms in more than 75 % of patients at 24 months. After 24 months a worsening of symptoms with deterioration occurs in 48-80 % [9, 10••, 12].

The clot is formed immediately after microfracture, followed by cell proliferation within the first days. The differentiation of progenitor cells in a hyaline-like cartilage is observed in the initial 3 weeks. However, after 36-42 weeks, this tissue becomes fibrocartilaginous and begins to suffer degeneration.

Research is being conducted to improve the quality of the tissue and prevent. These surveys try to act in several steps increasing the amount of mesenchymal cells, adding growth factors or providing a stabilization/protection of the clot formed through scaffolds or membranes [12–28].

Some articles highlight the small amounts of cells found after microfracture (less than 100 mesenchymal stem cells) [12], which would differentiate into chondrocytes and subsequent formation of a hyaline-like tissue. Aiming at a higher concentration of mesenchymal stem cells, studies suggest perforations with a larger diameter. They compared perforations of small diameter and large diameter, and showed a greater number of mesenchymal cells in the second group [29–31].

Another line of research aimed at a better environment for these migrated cells improve the proliferation/differentiation. For this, the clot is protected by biomembrane or scaffolds (natural or synthetic materials). The rational for the use of membranes is that the clot will not influence the surrounding environment and will not lose some of the cells to the articular cavity, thereby developing a better quality cartilage. A membrane protecting the clot improves the outcome of an isolated microfracture [14, 15, 20, 23, 24].

Some studies have shown that the application of hyaluronic acid with concomitant microfracture promotes chondroprotection and prevents degeneration of this new cartilage [13, 17, 25]. Also, adding diacerein to a microfracture is beneficial [13].

Thinking of a more biological response to microfracture, studies are adding growth factors, such as TGF-β, BMP, IGF-1, FGF, and PRP [18, 19, 21, 22, 26–28]. According to studies the application of PRP associated with microfracture can lead with better results than the microfracture as a single procedure [18, 19, 22, 26]. One single study did not find this evidence of better results with PRP [27].

Another way to improve the quality of this new cartilage is the application of stem cell/bone marrow concentrate [15–17, 24]. The intra-articular injection of rhFGF -18 (recombinant human fibroblastic growth factor 18) also promotes the improvement in the treatment of chondral lesions [21]. Zhang et al. [28] described the association between microfracture with BMP4 (bone morphogenetic protein- 4) and also found promising results.

It can be concluded that despite that microfracture is an established procedure performed worldwide, it is still evolving, seeking associations/technical changes to provide better functional outcomes and a better quality cartilage, especially in the long term.

Osteochondral allograft

Osteochondral allografts (OCA) are adaptable and constituted of 2 components, transplanting bone and cartilage from a donor patient into a recipient patient. The main advantage of using allograft is the presence of both viable hyaline cartilage and structural bone and can be designed for lesions with different shapes and contours [32].

OCA is a single stage peerless obtainable biologic option for salvage procedures following failed cell based repair, prior OCA transplantation for large chondral or osteochondral defects (ICRS Grade III–IV or >2–3 cm^2), or failed fixation of large, deep osteochondritis dissecans (OCD) lesions, extensive subchondral edema, or extensive bone loss that requires restoration [32, 33]. In older patients who are more sedentary and have low activity levels, synthetic total joint replacement is a viable option. The indications for OCA transplantation have been expanded to include biologic restoration of the knee joint for management of focal osteonecrosis, fracture malunion, joint restoration following tumor resection, and select cases of osteoarthritis [34].

The first intent of OCA application is to assure the composite of subchondral bone and articular cartilage with viable chondrocytes capable of maintaining metabolic activity with similar configuration and thickness as the surrounding native tissue following implantation [33]. To accomplish this point optimal storage conditions for the OCA must be achieved.

Storage and immunogenicity

The allograft chondrocytes must survive hypothermic storage and retain high amounts of viability to sustain the extracellular matrix. One study evaluated chondrocyte survival and material properties in a sheep condyle model and found a large drop-off in chondrocyte viability after 28 days of storage [35]. Other authors examined the effects of the lactated ringers or culture medium storage on chondrocyte viability. At 14 days, culture medium outperformed the lactated ringer's solution

(91 % vs 81 % chondrocyte viability [36]. The chondrocyte viability decreases in allografts stored for more than 14 days, and allografts generally should be implanted by 24 days [37].

A study provides a comparison of frozen with fresh allograft of goats regarding storage performance and chondrocyte viability. The fresh group showed a better cartilage stiffness and matrix content [38].

Recently, cryopreservation has been employed like vitrification of intact human articular cartilage on its bone base has been achieved with high index of cells viability (75 %) and reduced cytotoxic effects in of 10 mm diameter osteochondral dowels. The tissue was vitrified in liquid nitrogen for up to 3 months [39•].

Grafts stored at 37 °C had significantly better chondrocyte viability in the superficial and middle zones and reduced bone viability, which may diminish immunogenicity [40]. Based on this, the length of graft storage before transplantation could be increased with no deleterious effect on cellular viability of chondrocytes.

Surgical technique

Surgical technique for OCA varies based on specific characteristics of the lesion. The size of the articular cartilage defect may be underestimated on MRI [41]. For larger, asymmetric or more irregular femoral condylar lesions (mainly posterior location) and whole patella/large trochlea, a shell grafting technique can be employed. Usually fixation is necessary with bioabsorbable pins or compression screws [42].

For regular or contained cartilage lesion the press-fit plug technique can be recommended. Some allografts with dowel sizes available up to 35 mm in diameter can be chosen. The lesion is sized, and a guidewire is inserted into the center of the lesion, perpendicular to the articular surface. Usually, no additional fixation is necessary [43].

Depth and implant size should be considered in the operative setting. The influences of the depth-dependent inhomogeneity on the fluid pressurization, compressive stress enhance the fluid support to loading in the superficial zone by raising the fluid pressure and lowering the compressive effective stress. It also reduced the tensile stress and strain at the cartilage-bone interface [44]. One biomechanical study demonstrated that implant size and material properties have a significant effect on the failure of the fibrin that adhere the implant to the native tissue. Lack of anchorage to underlying bone, larger implant sizes, higher surface coefficient of friction, and higher compliance of the implant can increase the chance of implant loosening and delamination [45].

Clinical results

Current evidence suggests that between 74 % and 85 % of patients who underwent OCA experienced a subjective improvement [46, 47••]. One study with long-term follow-up 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 [46]. Levy et al. evaluated 129 knees with long-term follow-up demonstrated durable improvement in pain and function, with graft survivorship of 82 % at 10 years [47••].

Preparation

There are 3 variables that factor into the storage of osteochondral allografts: radiation, time, and temperature.

Irradiation of allograft tissue is utilized to decrease the risk of disease transmission in the host after transplantation. The radiation dose to eliminate viral DNA is 3–4 mRad, which kills chondrocytes and significantly decreases the graft's stiffness and strength [48]. For this reason, radiation is not used for fresh osteochondral allografts.

Regarding temperature, 3 types of storage techniques exist for osteochondral allografts: fresh-frozen, cryopreserved, and fresh. Fresh-frozen osteochondral allografts can be stored indefinitely at -80 °C and subsequently have a very low immunogenicity. The deep freezing, however, leads to very poor levels of chondrocyte viability (<5 %) in the articular cartilage portion of the grafts [49]. Although early research demonstrated improved angiogenesis and decreased immunogenicity in a mouse model with cryopreserved allografts, subsequent studies have shown poor chondrocyte viability that is limited to the superficial zone [50–52]. The low chondrocyte viability in both fresh-frozen and cryopreserved grafts led to the transplantation of only fresh osteochondral allografts [53]. After harvest and 24 hours of treatment in an antibiotic solution, fresh osteochondral allografts, sometimes referred to as fresh-refrigerated to differentiate from fresh-frozen grafts, are stored in either a lactated ringers solution or a physiologic culture medium at 4 °C. Based on the findings of these studies, tissue banks have converted to the use of nutritive culture medium for graft storage and current recommendations include implantation of fresh osteochondral allografts within 28 days of procurement. The extension of the time period for implantation has led to the terminology of fresh (up to 14 days) and prolonged-fresh (14–28 days) osteochondral allografts.

Autologous chondrocyte implantation

All types of procedures described above for the treatment of full thickness cartilage defect have their limitation and different outcomes. Due to this reason, techniques of tissue

engineering were developed attempting to better hyaline cartilage repair [6, 54–58].

Autologous Chondrocyte Implantation (ACI) is a procedure of tissue engineering and consists of 2 stages. The first stage consists of an arthroscopic biopsy of hyaline articular cartilage from an area of nonweightbearing. This cartilage is taken to a cell culture room, digested, and chondrocytes are extracted. Then they are expanded in culture. After expansion, the chondrocytes are implanted in the second stage procedure that consists of coverage of the defect by a periosteal flap and implantation of the chondrocytes underneath periosteal flap [6]. This procedure is called first generation of ACI. When using a collagen membrane instead of periosteal flap it is called second generation of ACI. When using a scaffold instead membrane or periosteal flap it is called third generation of ACI.

Although it is an obvious source of hyaline cartilage and good outcomes are seen, ACI also has some limitations. They are described as the small amount of hyaline cartilage obtained in a biopsy, the production also of a fibrocartilage repair, metabolic changes, and inactivity of diseased or older chondrocytes, and decreased function of the knee [59–63].

However, the limitations can be better addressed by changing culture media, centrifuge methods, culture in hypoxia, addition of growth factors to the culture, and others that contribute to the development of ACI techniques [64–68]. Although limitations may decrease as mentioned, the main complications are hypertrophy of the tissue repair, delamination, failure, not uniform distribution of cells in the repair area, and prolonged rehabilitation.

To date, tissue engineering is based on 3 main components: a cell source, a scaffold or stable matrix, and bioactive agents. The goal is that the hyaline cartilage produced in this procedure should be similar to the native cartilage regarding its composition of water, type II collagen, and proteoglycans. Even more, the repair tissue should occupy the entire defect and integrate to the native cartilage sharing the mechanical loads exerted to the joint [69].

In an attempt to evolve the cell therapy, mesenchymal stem cells may help. They are derived mainly from bone marrow tissue and have demonstrated high capacity of differentiation. It is also easier to obtain it compared with the biopsy of hyaline cartilage [70, 71••].

There are others source of mesenchymal stem cells as in adipose tissue, synovia, periosteum, muscle, and others [72–74]. As in ACI the use of stem cells also presents limitations, the quality of repair still not the same as in native articular cartilage due to quantitative and qualitative differences of the collagen [72, 74]. However, an observational cohort study demonstrated that clinical outcomes from ACI are comparable with bone marrow derived stem cells implantation [75]. It may be a future trend for cell therapy in full thickness cartilage defect because the stem cell transplantation required 1 less procedure, reduced costs, and minimize donor site morbidity [75].

Cell source seems to be the key to a good development of tissue engineering. The cells should be healthy, viable, easy access, easy to handle, nonimmunogenic, nontumorigenic. It is also important to have stable phenotype and adequate response to biological factors [76]. These cells may be acquired form an autologous or allogenous source. The first has the advantage to avoid immune response and disease transmission. The second can afford a higher number of cells without site morbidity [76].

The production of cartilage with its anabolic and catabolic process is influenced by signaling molecules. Cytokines, hormones, and growth factors are the most observed, as BMPs, IGF-1 and TGF-β that are of major importance followed by FGFs and EGF [77].

Scaffolds were developed to act in specific roles: to afford a niche to stimulate cartilage matrix production, to substitute temporarily the matrix while new matrix is being produced. The scaffolds also should mimic the effect of native cartilage, helping cell proliferation, differentiation, and interaction [78]. The 3D scaffolds are superior to the membranes because they facilitate arthroscopic procedure and are able to keep chondrocytes phenotype for a longer time [78].

Several techniques are described with membranes and scaffolds. The porcine type-I/III collagen bilayer seeded with chondrocytes also called as Matrix-Associated Chondrocytes Implantation (MACI) was the first development in the ACI technique showing promising results [79]. It also has shown superior outcomes when compared with microfracture [80] and good outcomes even in patients older than 40 years [81]. A hyaluronan-based scaffold that allows chondrocyte culture in a 3D manner was also described for clinical application with good outcomes for defect size larger than 1.5 cm^2 [82], this scaffold is called Hyalograft C and it is also described for 10 years follow-up with good outcomes [83].

An engineered construct of bone marrow cells and collagen membrane is recently being used as a 1-step cell implantation procedure. The bone marrow is harvested of the iliac crest and centrifuged to concentrate the cells from bone marrow, and then a clot is formed form the bone marrow aspiration and mixed with the cells. This clot fills the defect and a collagen membrane is used to cover by being anchored to the cartilage. This technique also has been described as a good procedure with good outcomes in a 3-years follow-up [84•]. It is important to say that the bone marrow cells are not only stem cells; other types of cells are also present.

Natural scaffolds may be based on proteins (collagen, silk) or based on carbohydrates (agarose, alginate, chitosan, and hyaluronan). Many of them present a large amount of water in its composition, so called Hydrogels. They are able to keep the round format of the chondrocytes as well as their phenotype [85, 86].

Synthetic scaffolds are also well known, mainly Polylactic Acid (PLA) and Polyglycolic acid (PGA). They have better mechanical resistance, increasing its integration capacity and load-bearing to the receptor site. However, the cell proliferation and phenotype maintenance are not satisfactory being the main disadvantage of these scaffolds [87].

Gene therapy associated with cell transplantation may be a future technique to be used. It has shown improvement in matrix cartilage repair [88, 89]. However, the technique to add it in cells brings a larger complexity. Immunogenicity is also concerning. Appropriated genes for this type of therapy may include IGF-1, TGF-β, BMP-2, BMP-7, and FGF-2 [88, 89].

Conclusions

The biological updates for full thickness cartilage defect are several. Improvements of standard surgical techniques are the most common updates. Microfracture associated with scaffolds, hyaluronic acid, PRP, and growth factors are important trend to the improvement of this surgical technique. The cryopreservation of allografts like vitrification may be a promisor change in this practice. The cell therapy has shown updates in 3D scaffolds as well as the use of mesenchymal stem cells instead chondrocytes. Gene therapy may also be a tool for cartilage repair in the future.

Compliance with Ethics Guidelines

Conflict of Interest Alexandre Pedro Nicolini, Rogerio Teixeira Carvalho, Bruno Dragone, Mario Lenza, Moises Cohen, and Mario Ferretti declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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