Chapter

Bio-Orthopedics: A New Approach to Osteoarthritis and Joint Disorders

Alberto Gobbi, Katarzyna Herman and Dawid Szwedowski

Abstract

Osteoarthritis is a major cause of functional limitation and a raising burden in aging population. Lately more research is directed into finding biological enhancement of healing processes in joint dysfunctions. Biological cell-based therapies for cartilage restoration treatment were created to address the need for the long-term viability of repaired tissues. Additionally, the use of biologic therapies is also considered in common disorders affecting ligaments and cartilage. However, if inevitable arthritic changes commence biological therapies offer options to delay the need for arthroplasty. This chapter provides insights into these regenerative, joint preservation techniques for cartilage treatment, osteoarthritis, and other joint disorders.

Keywords: cartilage, osteoarthritis, bio-orthopedics, PRP, BMAC, knee articular preservation

1. Introduction

Osteoarthritis (OA) is one of the most common joint diseases; characterized mainly by joint pain and functional impairment, due to cartilage degeneration, subchondral bone remodeling, and synovial inflammation [1]. OA affects 7% of the global population [2], making it an important problem to solve for the orthopedic surgeons.

Tissue healing is limited not only by the biochemical environment but also by other coexisting factors such as diabetes, smoking, hypercholesterolemia or local factors, which can further weaken healing [3]. Biological response has been studied for many years in order to optimize the healing processes. These have resulted in cell-based, cytokine-based, and scaffold-based therapies [4]. In this chapter, we analyze current concepts in the use of biological treatments. This book chapter intends to provide a review of the current status of biological therapies for OA and other joint disorders in orthopedics.

2. Biological options

2.1 PRP

Lately, research in OA has moved the attention toward biochemical pathways that can be aimed therapeutically through biological intervention. In the past decade, a great interest has been focused on platelet-rich plasma (PRP). It is one of the "hot topics" of regenerative medicine due to its potential to help in different conditions. PRP has surfaced as a biological therapy for the treatment of cartilage injuries and for intra-articular application to address knee pain. PRP contains cytokines, growth factors, and inflammatory mediators, all capable of stimulating cartilage, subchondral bone, and soft tissue healing [5]. PRP contains a significant number of growth factors and proteins in the alpha granules of platelets, that were observed to have regenerative and analgesic effect [6, 7]. What is more, studies demonstrated that platelet-rich plasma (PRP) has an anti-inflammatory influence and counteracts catabolic processes within the joint [8–10]. Additionally, it has been shown to promote chondrocyte proliferation and increase the synthesis of collagen and proteoglycans and for this reason, the application of PRP for osteochondral pathologies has increased [5, 11]. PRP can be categorized as leucocyte-rich PRP (L-PRP), leucocyte-poor PRP (LP-PRP), or platelet-poor plasma (PPP) depending on the preparation technique. The ingredients of PRP categories vary, depending on which system is used to prepare the autologous blood, so care must be taken when choosing the method. There is no one gold standard, so injections of PRP can be done once or in cycles of 3 or more injections. PRP is acquired from patients' peripheral blood. The venous blood is centrifuged in a special probe, according to instructions provided by the manufacturer. When using intra-articularly the PRP is injected into the joint after careful skin disinfection at the needle entry point. The highest beneficial effect is seen at 6 months after cycle of injections, however it may last up to two years [12]. Authors of a meta-analysis of randomized controlled trials using PRP in treatment of knee OA concluded that a statistically significant beneficial effect over placebo was observed at 6 and 12 months [13]. It was also shown that PRP is more effective both in short- or long-term pain and functional recovery when compared to HA [14].

2.2 Hyaluronic acid

HA is a non-sulphated glycosaminoglycan that consists of D-glucuronic acid and N-acetylglucosamine units. It ensures tissue hydration due to its hydrophilic properties and high solubility in an aqueous environment [15]. In a healthy joint it is produced by the synovium, exactly the type B synoviocytes and fibroblast. Its role is to preserve the viscoelastic and functional characteristics of the articular cartilage [16] in the same time promoting chondrocytes proliferation and differentiation [17]. Additionally, it provides joint lubrication and shock absorption. Interestingly in a study HA was shown to inhibit tissue nociceptors [16]. HA also inhibits IL-1β-induced oxidative stress and the inflammatory mediators, such as metalloproteases (MMP-13), nitric oxide (NO), and prostaglandin (PGE2) [18]. Various HA compounds are available that differ in molecular weight (MW), composition, and dosage regimens. High MW HA consists of molecules ranging from 3000 kDa to 6000 kDa; medium MW from 1500 to 3000 kDa; low MW has been described as ≤1500 kDa. High molecular weight was proven to increase the fluid retention into the joint and to have greater anti-inflammatory effect than low MW HA [19]. Clearance rate of HA is another important factor influencing its effectiveness. That is why stabilizers have

been introduced to slow down the clearance rate. One of the used options is trehalose, a disaccharide that acts as a protector of HA. In an in vitro model hyaluronic acid combined with trehalose had improved resistance to hyaluronidase enzyme compared to standard therapy [20]. Additionally in a randomized controlled trial comparing 1% trehalose hyaluronic acid (T-HA) versus non-trehalose hyaluronic acid (NT-HA) for patients with OA KOOS, VAS and IKDC improved for T-HA group at 6 months post-injection while for NT-HA patients it decreased to baseline. The difference was reported statistically significant ($P < 0.05$) [21]. At our institution we are currently conducting two clinical trials on the use of new formulations of HA for the treatment of knee OA. Particularly, one consists of a high chain (1800–2600 KDalton) combined with Niacinamide, an excipient that protects the acid from hyaluronidase degradation activity, promoting longer HA stability. Our preliminary results confirm a more durable effect of HA with Niacinamide compared to standard HA in patients affected with knee OA, at 6-month follow-up. We are also evaluating the efficacy of a second product of HA combined with Collagen type I which reinforces the cartilaginous matrix deteriorated by the ongoing pathological processes of osteoarthritis. We are testing it in young athletic patients to evaluate if they can have more benefits when compared with other therapies.

2.3 Adipose stem cells

An Autologous Microfragmented Adipose Tissue (MFAT) and Stromal Vascular Fraction (SVF) in contrast to peripheral blood has 25,000 times more reparative cells [22]. MFAT is acquired from adipose tissue from abdominal or supragluteal area with a special lipoaspirate cannula. Lipoaspirate is transferred to the special low-pressure cylindrical system and combined with saline solution and then mixed. A final product is a concentration of pericytes and MScs. This is then applied into the joint through an injection or during arthroscopy. Promising results have been shown in literature. In multi-centric, international, open-label study evaluating the outcomes of MFAT injections in patients with knee OA at 2-year follow-up significant functional and quality of life improvement was seen in 88% of patients [23]. In a prospective randomized control trial comparing leukocyte-poor PRP combined with HA and MFAT no significant superiority was seen for either group, both procedures were found to be safe with no major complications showing good results at mid-term follow-up [24]. Additionally, a new technique using SVF and platelet-rich plasma to treat knee osteoarthritis (OA) has been recently described. First, the adipose tissue is harvested from abdominal area with the harvester cannula connected to syringe (**Figure 1**). Meanwhile, leucocyte-poor platelet-rich plasma (LP-PRP) preparation is performed. The harvested fat is transferred to an ACP double syringe for first-round centrifugation with an end product of a middle fraction that is the condensed fatty tissue containing the stromal vascular fraction (SVF) (**Figure 2**). After fragmentation the lipoaspirate is transferred back to the ACP double syringe for second-round centrifugation. After centrifugation the SVF fraction is retrieved. Lastly the SVF is combined with LP-PRP injected into the patient's knee joint (**Figure 3**) [25].

2.4 Bone marrow aspirate

MSCs have multi-differentiation ability, which is why they have been identified as an attractive cell source to regenerate tissue. MSC represents only 0.0001 to 0.01%

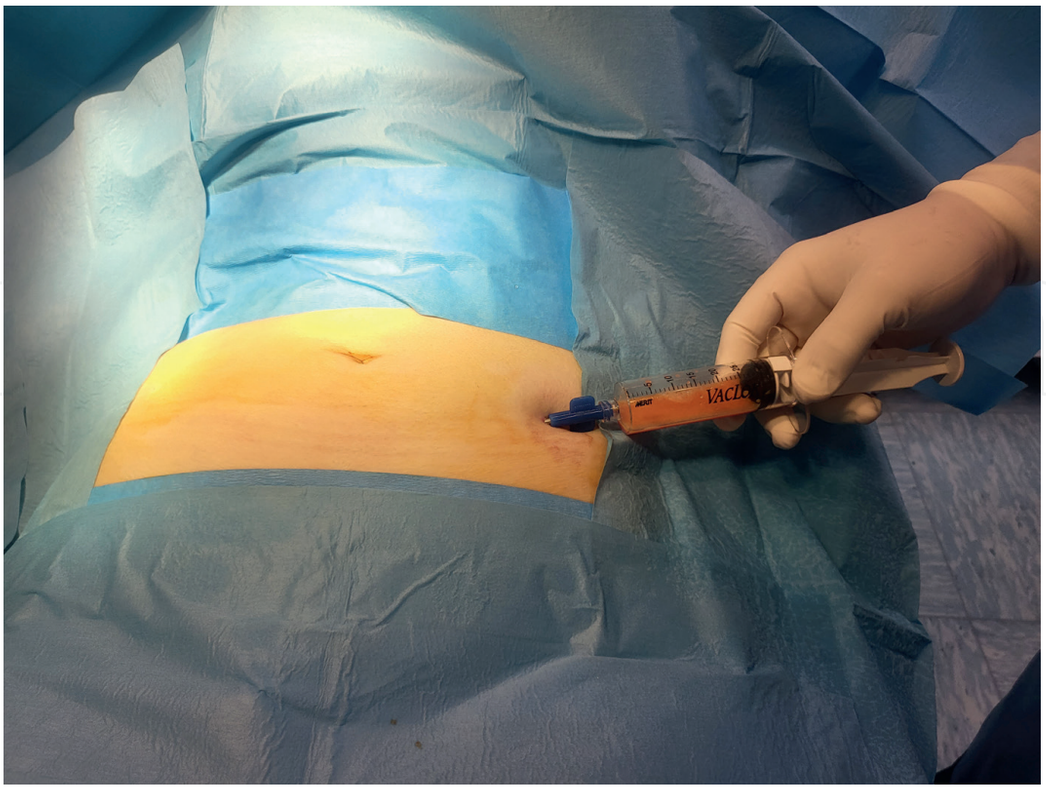

Figure 1.
Lipoaspiration with a 2.1 mm × 15 cm harvester cannula connected to syringe with Johnnie snap locking system through 5 mm skin cut after infiltrating the fatty tissue with a tumescent solution.

mononuclear cells in bone marrow aspirates [26]. Bone marrow aspirate concentrate (BMAC) consists of variety of growth factors such as the platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and bone morphogenetic proteins (BMP)-2 and BMP-7, known to have anabolic and anti-inflammatory effects [27]. Hypothetically MSCs, rather than contributing to tissue formation, act as site-regulated "drugs stores" by releasing immunomodulatory factors activated by local injury [28].

BMAC with a Hyaluronic scaffold (Hyaff) has been used to treat chondral lesions of the knee with good clinical outcomes at long-term follow-up using [29–31]. This treatment was not only successful for single lesions but also in cases of multiple compartment injury, extensive lesions, or in older patients [30, 32, 33]. The composition of BMAC and what could be its mechanism of action has been a point of interest lately. Preliminary data seem to indicate there was no correlation between the clinical outcome and the number of Colony Forming Units (CFU; indirect estimation of the number of MSCs) found in bone marrow aspirate [29]. Interestingly, bone marrow aspirate harvested with Marrow Cellution system was shown to contain a relatively high CFU-fs/mL and CD34+/mL and therefore not requiring centrifugation. The level of CFU-fs/mL was significantly higher in comparison to BMAC in side-by-side evaluation from the same patient [34].

Although the use of BMAC shows good effects in cartilage repair surgery, its use as an injectable therapy is not so common. As a recent RCT has shown, there was no superiority of BMAC over PRP in knee OA therapy [35]. Though BMAC is one of the most appealing sources for cartilage defect repair, several aspects such as safety, amount of aspirate, and scaffold requirement require further investigation.

Figure 2.
Lipoaspirate transferred into ACP double syringe.

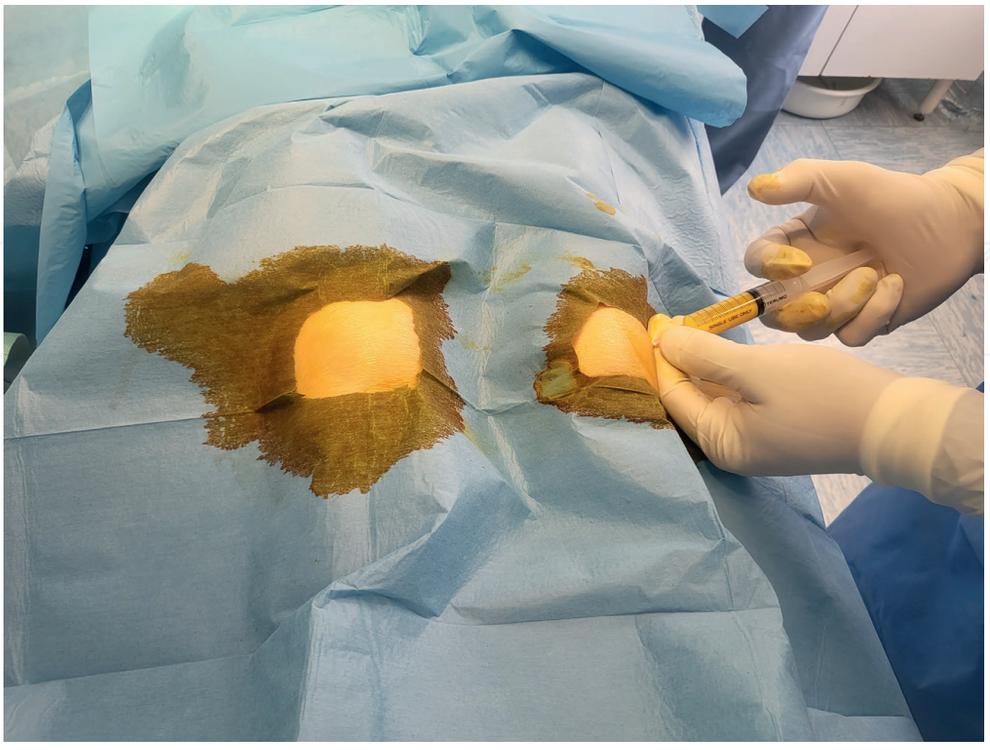

Figure 3.
Injection of the final product, 5 mL of leucocyte-poor platelet-rich plasma (LP-PRP) and 3 mL of SVF in a 10-mL syringe.

3. Applications

3.1 Ligament repair

After a ligament is injured, a cascade of processes begins, it is characterized by cellular proliferation, migration, and collagen production. However, the process of platelet fibrin clot formation is significantly deficient in the cases of intra-articular injury [36]. Synovial fluid has been shown to reduce ACL fibroblast proliferation and migration, thus delaying tissue healing [37]. What is more, the presence of circulating plasmin in the joint space has been suggested to be the cause of suboptimal clot formation [38]. Without clot, the torn ligamentous fibers remain separated after injury and subsequent tissue repair is impaired.

PRP contains growth factors that enhance the processes related to cellular activity and their arrival at the area of the lesion. Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) stimulate cell population's activation, migration, and proliferation. Fibroblast growth factor (FGF) is vital in collagen synthesis stimulation and fibroblast proliferation, both important elements in the tendon structure [39]. Due to these biological properties, PRP has been suggested to enhance the healing processes in ligaments.

For example, one study showed that the addition of PRP to the sutured ACL repairs did not improve AP knee laxity, maximum tensile load, or linear stiffness of the ACL after 14 weeks in vivo, compared to ACL repair without the addition of PRP [40]. However, when used with a collagen-PRP scaffold, the same author reported a significant improvement in load at yield, maximum load, and linear stiffness at four weeks. The PRP effect alone in ACL repair remains debatable, with significant graft maturation enhancement over time but no clear benefits on clinical outcomes [39].

Mixed results were also found with PRP administration in MCL lesions. LaPrade et al. described that one dose of either PPP or increasing twice the amount of PRP injected at the time of injury did not enhance ligament healing [41]. Furthermore, authors found that a four-time fold increase in the administered dose of PRP showed a significant adverse outcome on ligament strength six weeks after injury. In contrast, in a biomechanical analysis study by Yoshioka et al., there was a significant increase in the structural properties of MCLs in rabbits given leukocyte-poor PRP relative to controls [42].

Other cell therapies reported for ligament healing enhancement included dermal fibroblasts, which were shown to have promising properties in an in vivo models [43]. Adult MSCs represent a known cellular therapy used for tendon engineering; these cells' self-renewal capacity and multi-lineage differentiation potential have become common treatment. Bone marrow stromal cells embedded on polylactide or glycolide sutures have shown higher collagen production and DNA content than sutures seeded with anterior cruciate ligament fibroblasts and skin fibroblasts [41].

Combinations of cellular isolates and scaffolding have a promising role in treating tendon and ligament injury. In a prospective case series involving 50 patients (mean age 28.3 years), it was shown that ACL primary repair combined with bone marrow stimulation and BMAC-Hyaluronic acid bioabsorbable scaffold is an efficient method to restore knee stability and function in young athletes with acute partial ACL tears, after 10-year of follow-up [44, 45].

3.2 Meniscal repair

Even though techniques of repair have changed, many new instruments have been developed, still in the literature the failure rate of meniscus repair is reported to be around 20–25% [46]. Biological healing enhancement for the meniscal lesions treatment may be one of possible options to alter the outcomes [47].

Possible options for biological enhancement of menisci healing include rasping, needling, using a fibrin clot, platelet-rich plasma, bone marrow aspirate, and a scaffold with bone marrow addition [48].

In the 2019 ESSKA consensus on traumatic meniscus tears rasping, needling, and fibrin clot weak evidence was reported [48]. Although similar conclusion was reached in case of PRP use, a study that was not included in the consensus shown promising results. A prospective, randomized, double-blind, placebo-controlled trial with 37 patients with unstable complete vertical longitudinal tears, half treated with repair and placebo and half with repair and PRP. Same suturing all-inside technique was used. At 18-month follow-up the healing rate of tears was superior in the PRP-treated group (85% versus 47%) [49].

In another study on 550 patients treated for meniscal tears, authors found that the use of PRP resulted in improved survival of isolated meniscal repairs, but had no effect on survival of meniscal repairs with coexisting ACL reconstruction [50].

Bone marrow represents a great potential in healing enhancement. A prospective, randomized, double-blind, parallel-group, placebo-controlled study analyzing complete vertical meniscus tears in 40 patients randomized into two groups both using same suturing technique but one with addition of bone marrow venting procedure. Interestingly, the authors found a significant improvement of healing rate (rated by Second-Look Arthroscopy) in the bone marrow venting group compared to repair alone [51]. Another interesting study by Piontek et al. involved the use of meniscus suture with a collagen membrane wrapping together with bone marrow aspirate to treat combined and complex meniscal tears. They found a statistically significant improvement in subjective scores between the preoperative, 2-year follow-up, and 5-year follow-up [52, 53].

3.3 Healing the cartilage

Bone marrow stimulation techniques refer to methods using bleeding from subchondral marrow space and further formation of fibrin clot, which functions as scaffold for subchondral stem cell migration and consequent formation of fibrocartilage. In general, full-thickness cartilage lesion of a surface area < 1 cm^2 is considered an indication for a bone marrow stimulation technique as an isolated procedure [54]. Although, one should be aware that these recommendations should be carefully considered for every patient individually.

Microfracture is most commonly known and used procedure due to availability, simplicity, and small cost [55–57]. The lesion should be prepared, loose cartilage should be removed, and borders made perpendicular to subchondral bone and then holes should be made with an arthroscopic awl. As the saline pressure is lowered, the release of fat droplets and the bleeding begins which will later form a clot on the defect [56, 58, 59]. Studies have shown that this fibrocartilage matrix consists mainly of type I collagen and other non-collagenous proteins, making this tissue more delicate and less elastic, that is why it is common for the initial satisfactory results to deteriorate over long term [60–62]. Better results may be expected in younger patients with smaller lesions. This technique should be used cautiously as it may damage the

subchondral bone and lead to the formation of microcysts, therefore, compromising the articular surface for future procedures [63].

Autologous Matrix-Induced Chondrogenesis (AMIC) is based on the same idea as microfracture but supported with a porcine collagen scaffold [64]. This technique is indicated for focal chondral or osteochondral defects, Outerbridge classification grade 3–4 with a defect size of 1.0–8.0 $cm^{2,}$ and patient age of 18 to 55 years old [3]. After the defect is treated with microfracture a scaffold is added to cover the lesion and to allow the ingrowth of mesenchymal stem cells (MSCs) from the subchondral bone. The main advantage of the AMIC procedure is no donor site morbidity and the possibility of arthroscopic approach. The procedure is also inexpensive compared to autologous chondrocyte implantation (ACI). Good clinical results of AMIC in mid-term follow-ups have been described [65].

In acute lesions, use of autologous cartilage is an optimal alternative to repair a cartilage defect. It is described that covering an acute cartilage defect with minced fragments from a large piece of cartilage achieves good clinical results [66]. In this technique a large chondral fragment is minced into multiple small ones (<1 x 1 x 1 mm) with a scalpel. First, the cartilage lesion is debrided and drilled into the subchondral bone using a 1.4 mm K-wire. Then, minced cartilage fragments are placed into the defect and attached using fibrin glue. This concept is known since 1980s. The procedure using minced cartilage was modified and combined with various materials to become Cartilage Autograft Implantation System (CAIS) [67]. A cartilage paste (smaller cartilage size) was demonstrated to significantly increase extracellular matrix production [68]. Recently 10–23-year long-term results were reported in 74 patients cured with Articular Cartilage Paste Graft, the biopsies of the healed tissue revealed that 14 (48.3%) contained hyaline-like cartilage, 24 (82.8%) fibrocartilage with GAG, 10 (34.5%) fibrocartilage without GAG, and 3 (10.3%)fibrous tissue [69].

Osteochondral Autograft Transfer System (OATS) may be done in a single-stage procedure, arthroscopically or through arthrotomy. Cylindrical plugs are collected from donor sites from non-articulating regions. The plug consists of not only cartilage but also the subchondral bone, that is why it may recreate the osteochondral unit in cartilage lesions with damaged subchondral bone. This method is one of the few that has the advantage of restoring the hyaline cartilage. OATS is usually used for lesions smaller than 2 cm^2. In a 17-year prospective multicentric study performed in 383 found good to excellent results in 91% of femoral mosaicplasty, 86% of tibial, and 74% of patellofemoral mosaicplasty [70]. However, Wu et al. have shown that osteochondral plugs protruding 1 mm caused drastically increased contact pressures within the joint. Additionally, treatment using the OAT technique is restricted by the availability of autologous tissue, as donor site morbidity is a concern if multiple grafts are harvested.

Fresh osteochondral allograft is used mostly in lesions where OATS cannot be performed. The advantages of using allografts include the plasticity of graft sizing and the chance to treat the entire lesion with a one transplanted plug and no donor site morbidity. Some disadvantages include lower chondrocyte viability due to storage and managing and potential immunogenic response concerns.

ACI consists of two steps: first, a piece of healthy cartilage is obtained from a non-weight bearing area and subsequently expanded in vitro. The second step is grafting of the chondrocyte suspension into the defect [57]. Four generations of ACI have been introduced through the years. The first generation [71] is based on infusion of the chondrocyte suspension under periosteal flap, while in second generation the chondrocyte suspension is injected under a collagen membrane. The third generation also known as matrix-induced autologous chondrocyte implantation (MACI)

is a combination of the expanded chondrocytes embedded on a scaffold which is implanted in the cartilage defect. The fourth generation which is a one-step procedure with chondrocyte isolation through biopsies and direct implantation. Long-term results with ACI first-generation method were published with good term results at 20-year follow-up [72]; However, some studies report significantly better functional outcomes in patients who underwent second-generation ACI compared to patients with first-generation ACI [73, 74] ACI in comparison with bone marrow stimulating techniques such as microfracture has shown to be superior to time due to longer-lasting effects. Although the final tissue is still fibrocartilage, it is more "hyaline-like" in contrast to the one after microfracture procedure [32, 75]. ACI has proven a durable solution in treatment of large full-thickness cartilage lesions, but the need for two surgical interventions, the cost of chondrocyte culture, and equal results compared to one-step biological scaffolds stay the ACI technique's major drawbacks.

HA-BMAC developed 30 years ago, allows the treatment of larger cartilage lesions in a one-step surgery. This method provided good long-term results [29, 30, 32, 33] and demonstrated its superiority to microfracture. Additionally, it can be used in multiple compartment and extensive lesions or in older patients [30, 32, 33]. The senior authors' selected method is a one-step cartilage repair with a three-dimensional hyaluronic acid-based scaffold "Hyalofast" (Anika Therapeutics, Bedford, Massachusets, U.S.) combined with activated bone marrow aspirate concentrate (HA-BMAC). Contrasted with two-step MACI, the clinical outcomes have not shown a significant difference and also there was no relationship between the clinical outcome and the number of Colony Forming Units (CFU) found in bone marrow aspirate [29].

Depending on the lesion's extent and location the procedure is done through a small arthrotomy or arthroscopically. Loose cartilage is removed, vertical walls are made around the periphery of the defect with special chondrectomes. Then the calcified cartilage layer must be thoroughly removed without damaging the subchondral plate (**Figure 4A**). The defects are measured with aluminum foil templates which are later used to cut out a matching hyaluronic acid scaffold. Bone marrow is collected from the iliac crest and centrifuged to obtain a concentrated bone marrow which is later mixed with Batroxobin (Plateltex®act-Plateltex S.R.O. Bratislava, SK) to create a clot. The hyaluronic acid-based scaffold and clot are combined to create a biologically active structure for cartilage repair. The HA-BMAC is placed on the lesion and secured with fibrin glue (**Figure 4B**). Afterward, the knee is cycled to check stability [63]. Sadlik et al. demonstrated a variation of this technique using morselized bone graft to fill the lesion and then covered with hyaluronic acid scaffold embedded with BMAC [76].

3.4 Healing the subchondral bone (OCP)

BMAC may as well be used to treat subchondral bone pathologies. Cartilage and subchondral bone work as a unit and over the last few years a debate on the function of subchondral bone has been going on. Bone marrow lesions (BMLs) are the focal defects in the subchondral bone and can be identified by magnetic resonance imaging (MRI). Number of pathologies may be causing such lesions with an ischemic, mechanical or reactive background. When assessing a patient with BML it is vital to evaluate whether the lesion is reversible and irreversible [77]. Both biological and mechanical improvement of osteochondral unit can be achieved with Osteo-Core-Plasty (Marrow Cellution™) a minimally invasive subchondral bone augmentation. This technique may also be used to treat insufficiency fractures, subchondral cysts, and osteonecrosis [78].

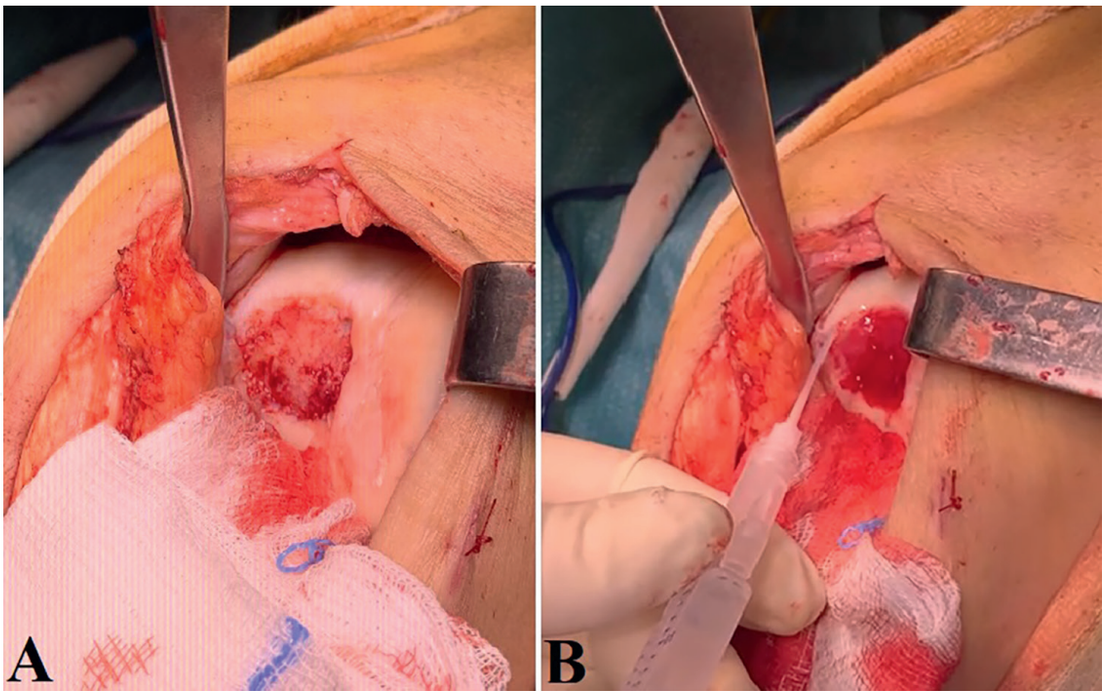

Figure 4.
(A) chondral defect on the patella after removal of loose cartilage and a calcified layer of subchondral bone with perpendicular borders of the lesion prepared for scaffold implantation. (B) The lesion after implantation of the HA-BMAC secured with fibrin glue.

This method is made of two parts, first being the aspiration of bone marrow and second application of the material into the defect. Bone marrow is aspirated from the iliac crest. Application may be done arthroscopically or through an open approach, both need fluoroscopic assistance. Necrotic Tissue Zone is recognized under fluoroscopy on AP and lateral images. In an open technique the lesion is debrided, and necrotic bone underneath is separated and removed. In an arthroscopic technique a K-wire is put to target zone from outside the joint and a cannulated drill bit is inserted over the K-Wire. Then Marrow Cellution Bone Core Graft is delivered to the necrotic zone with Extraction/Delivery Tool in both open and arthroscopic approach. The bone core graft is pressed with a probe to aim point. Finally, aspirated Marrow Cellution™ is injected to the necrotic site or in case of an open procedure the Marrow Cellution™ Saturated Matrix Scaffold Membrane is used [79]. In Osteo-Core-Plasty, there is no need for centrifugation and the surgeon can apply the aspirate to the target zone [80].

4. Conclusion

OA is a raising problem and many opportunities to treat cartilage lesions and early OA have been reported. Cell therapies using chondrocytes, MSCs, and other cell sources have been used to treat joint pathologies. In order to obtain the best cartilage quality, these cartilage preserving/regenerating methods combined with addressing coexisting intraarticular pathologies or limb alignment issued. The biology of the articular cartilage must be fully understood before cartilage repair technologies can advance further. Collecting evidence of experimental studies on novel techniques for biological healing is vital to advance the treatment possibilities for patients. Therefore, use of the most appropriate line of treatment and proper patient selection is key to improving results.

Bio-Orthopedics: A New Approach to Osteoarthritis and Joint Disorders
DOI: http://dx.doi.org/10.5772/intechopen.110845

IntechOpen

Author details

Alberto Gobbi*, Katarzyna Herman and Dawid Szwedowski
O.A.S.I. Bioresearch Foundation Gobbi N.P.O., Milan, Italy

*Address all correspondence to: gobbi@cartilagedoctor.it

IntechOpen

References

[1] Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: A lancet commission. The Lancet. 2020;**396**(10264):1711-1712

[2] Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) results. 2020. [Internet]. [cited 2021 Aug 10]. Available from: http://ghdx.healthdata.org/gbd-results-tool. [Accessed: August 8, 2020]

[3] Gobbi A, Lane JG, Espregueira-Mendes J, Karahan M. Bio-Orthopaedics: A New Approach. Bio-orthopaedics: A New Approach; 2017. pp. 1-696

[4] Herman K, Gobbi A. Evidence-based approach to Orthobiologics for osteoarthritis and other joint disorders. Physical Medicine and Rehabilitation Clinics of North America. 2023;**34**(1):71-81

[5] Salamanna F, Veronesi F, Maglio M, Della Bella E, Sartori M, Fini M. New and emerging strategies in platelet-rich plasma application in musculoskeletal regenerative procedures: General overview on still open questions and outlook. BioMed Research International. 2015;**2015**:846045. DOI: 10.1155/2015/846045. E

[6] Ulrich-Vinther M, Maloney MD, Schwarz EM, Rosier R, O'Keefe RJ. Articular cartilage biology. Journal of the American Academy of Orthopaedic Surgeons. 2003;**11**(6):421-430

[7] Asfaha S, Cenac N, Houle S, Altier C, Papez MD, Nguyen C, et al. Protease-activated receptor-4: A novel mechanism of inflammatory pain modulation. British Journal of Pharmacology. 2007;**150**(2):176-185

[8] Sánchez M, Anitua E, Delgado D, Sanchez P, Prado R, Goiriena JJ, et al. A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of platelet rich plasma. Expert Opinion on Biological Therapy. 2016;**16**(5):627-643

[9] Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: A prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. The American Journal of Sports Medicine. 2017;**45**(2):339-346

[10] Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clinical Orthopaedics and Related Research. 2011;**469**(10):2706-2715 [Internet] [cited 2023 Feb 25] Available from: https://pubmed.ncbi.nlm.nih.gov/21403984/

[11] Lee CH, Cook JL, Mendelson A, Moioli EK, Yao H, Mao JJ. Regeneration of the articular surface of the rabbit synovial joint by cell homing: A proof of concept study. Lancet. 2010;**376**(9739):440-448

[12] Gobbi A, Lad D, Karnatzikos G. The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee. Knee Surgery, Sports Traumatology, Arthroscopy. 2015;**23**(8):2170-2177 [Internet] [cited 2023 Nov 18] Available from: https://pubmed.ncbi.nlm.nih.gov/24748286/

[13] Filardo G, Previtali D, Napoli F, Candrian C, Zaffagnini S, Grassi A. PRP injections for the treatment of knee osteoarthritis: A meta-analysis of randomized controlled trials. Cartilage. 2021;**13**(1):364S-375S

[14] Tang JZ, Nie MJ, Zhao JZ, Zhang GC, Zhang Q, Wang B. Platelet-rich plasma versus hyaluronic acid in the treatment of knee osteoarthritis: A meta-analysis. Journal of Orthopaedic Surgery and Research. 11 Sep 2020;**15**(1):403. DOI: 10.1186/s13018-020-01919-9

[15] Santilli V, Paoloni M, Mangone M, Alviti F, Bernetti A. Hyaluronic acid in the management of osteoarthritis: Injection therapies innovations. Clinical Cases in Mineral and Bone Metabolism. 2016;**13**(2):131

[16] Waddell DD, Bert JM. The use of Hyaluronan after arthroscopic surgery of the knee. Arthroscopy - Journal of Arthroscopic and Related Surgery. 2010;**26**:105-111

[17] Watterson JR, Esdaile JM. Viscosupplementation: Therapeutic Mechanisms and Clinical Potential in Osteoarthritis of the Knee. Journal of the American Academy of Orthopaedic Surgeons. Sep 2000;**8**(5):277-284

[18] Mongkhon JMM, Thach M, Shi Q, Fernandes JC, Fahmi H, Benderdour M. Sorbitol-modified hyaluronic acid reduces oxidative stress, apoptosis and mediators of inflammation and catabolism in human osteoarthritic chondrocytes. Inflammation Research. 2014;**63**(8):691-701

[19] Bagga H, Burkhardt D, Sambrook P, March L. Longterm effects of Intraarticular Hyaluronan on synovial fluid in osteoarthritis of the knee. The Journal of Rheumatology. 2006;**33**:5

[20] Giardina S. In vitro efficacy study: Evaluation of susceptibility of a hyaluronate- based product to the activity of the hyaluronidase. Report number E.IT.076_2018/2116 B. performed by Complife group. 2018.

[21] Gobbi A, Morales M, Avio G, D'Ambrosi R. Double-blinded prospective randomized clinical trial in knee joint osteoarthritis treatment: Safety assessment and performance of trehalose hyaluronic acid versus standard infiltrative therapy based on medium-weight sodium hyaluronate. Journal of Cartilage & Joint Preservation. 2022;**2**(3):100043. [Internet] Available from:. DOI: 10.1016/j.jcjp.2022.100043

[22] Russo A, Screpis D, di Donato SL, Bonetti S, Piovan G, Zorzi C. Autologous micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: An update at 3 year follow-up. Journal of Experimental Orthopaedics. 19 Dec 2018;**5**(1):52. DOI: 10.1186/s40634-018-0169-x

[23] Gobbi A, Dallo I, Rogers C, Striano RD, Mautner K, Bowers R, et al. Two-year clinical outcomes of autologous microfragmented adipose tissue in elderly patients with knee osteoarthritis: A multi-centric, international study. International Orthopaedics. 2021;**45**(5):1179-1188 [Internet] [cited 2023 Mar 6] Available from: https://pubmed.ncbi.nlm.nih.gov/33649891/

[24] Gobbi A, Dallo I, D'Ambrosi R. Autologous microfragmented adipose tissue and leukocyte-poor platelet-rich plasma combined with hyaluronic acid show comparable clinical outcomes for symptomatic early knee osteoarthritis over a two-year follow-up period: A prospective randomized clinical trial. European Journal of Orthopaedic Surgery and Traumatology. 2022. Available from: https://link.springer.com/article/10.1007/s00590-022-03356-2#article-info

[25] Dallo I, Morales M, Gobbi A. Platelets and adipose stroma combined for the treatment of the arthritic knee. Arthroscopy Techniques. 2021;**10**(11):e2407 [Internet] [cited

2023 Mar 6] Available from: http://pmc/articles/PMC8626615/

[26] Martin DR, Cox NR, Hathcock TL, Niemeyer GP, Baker HJ. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Experimental Hematology. 2002;**30**(8):879-886

[27] McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. Journal of Orthopaedic Research. 2009;**27**(8):1033-1042

[28] Caplan AI. Mesenchymal stem cells: Time to change the name! Stem Cells Translational Medicine. 2017;**6**(6):1445-1451

[29] Gobbi A, Karnatzikos G, Scotti C, Mahajan V, Mazzucco L, Grigolo B. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: Results at 2-year follow-up. Cartilage. 2011;**2**(3):286-299 [Internet] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4300809&tool=pmcentrez&rendertype=abstract

[30] Gobbi A, Scotti C, Karnatzikos G, Mudhigere A, Castro M, Peretti GM. One-step surgery with multipotent stem cells and Hyaluronan-based scaffold for the treatment of full-thickness chondral defects of the knee in patients older than 45 years. Knee Surgery, Sports Traumatology, Arthroscopy. Aug 2017;**25**(8):2494-2501

[31] Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture: Five-year follow-up. The American Journal of Sports Medicine. 2016;**44**(11):2846-2854 [Internet] [cited 2022 Oct 15] Available from: https://pubmed.ncbi.nlm.nih.gov/27474386/

[32] Gobbi A, Chaurasia S, Karnatzikos G, Nakamura N. Matrix-induced autologous chondrocyte implantation versus multipotent stem cells for the treatment of large patellofemoral chondral lesions: A nonrandomized prospective trial. Cartilage. 2015;**6**(2):82-97

[33] Gobbi A, Karnatzikos G, Sankineani SR. One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. American Journal of Sports Medicine. 2014;**42**(3):648-657

[34] Scarpone M, Kuebler D, Chambers A, De Filippo CM, Amatuzio M, Ichim TE, et al. Isolation of clinically relevant concentrations of bone marrow mesenchymal stem cells without centrifugation 11 medical and health sciences 1103 clinical sciences. Journal of Translational Medicine. 2019;**17**(1):1-10

[35] Anz AW, Plummer HA, Cohen A, Everts PA, Andrews JR, Hackel JG. Bone marrow aspirate concentrate is equivalent to platelet-rich plasma for the treatment of knee osteoarthritis at 2 years: A prospective randomized trial. The American Journal of Sports Medicine. 2022;**50**(3):618-629 [Internet] [cited 2022 Oct 16] Available from: https://pubmed.ncbi.nlm.nih.gov/35289231/

[36] Murray MM, Martin SD, Martin TL, Spector M. Histological changes in the human anterior cruciate ligament after rupture. Journal of Bone and Joint Surgery. 2000;**82**(10):1387-1397

[37] Rość D, Powierza W, Zastawna E, Drewniak W, Michalski A, Kotschy M.

Post-traumatic plasminogenesis in intraarticular exudate in the knee joint. Medical Science Monitor. 2002;**8**(5):CR371-8

[38] Andrish J, Holmes R. Effects of synovial fluid on fibroblasts in tissue culture. Clinical Orthopaedics and Related Research. 1979;**138**:279-283

[39] di Matteo B, Andriolo L, Filardo G, Loibl M, Zellner J, Koch M, et al. Biologic agents for anterior cruciate ligament healing: A systematic review. World Journal of Orthopedics. 2016;**7**(9):592-603

[40] Murray MM, Palmer M, Abreu E, Spindler KP, Zurakowski D, Fleming BC. Platelet-rich plasma alone is not sufficient to enhance suture repair of the ACL in skeletally immature animals: An in vivo study. Journal of Orthopaedic Research. 2009;**27**(5):639-645

[41] LaPrade RF, Goodrich LR, Phillips J, Dornan GJ, Turnbull TL, Hawes ML, et al. Use of platelet-rich plasma immediately after an injury did not improve ligament healing, and increasing platelet concentrations was detrimental in an In vivo animal model. American Journal of Sports Medicine. 2018;**46**(3):702-712

[42] Yoshioka T, Kanamori A, Washio T, Aoto K, Uemura K, Sakane M, et al. The effects of plasma rich in growth factors (PRGF-Endoret) on healing of medial collateral ligament of the knee. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;**21**(8):1763-1769

[43] van Eijk F, Saris DBF, Riesle J, Willems WJ, van Blitterswijk CA, Verbout AJ, et al. Tissue engineering of ligaments: A comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. Tissue Engineering. 2004;**10**(5-6):893-903 [Internet] [cited 2022 Oct 7] Available from: https://www.liebertpub.com/doi/10.1089/1076327041348428

[44] Gobbi A, Whyte GP. Biological Augmentation in Acute ACL Repair. Bio-Orthopaedics: A New Approach. 2017. pp. 325-35. [Internet] [cited 2022 Oct 7] Available from: https://link.springer.com/chapter/10.1007/978-3-662-54181-4_25

[45] Murray MM, Flutie BM, Kalish LA, Ecklund K, Fleming BC, Proffen BL, et al. The bridge-enhanced anterior cruciate ligament repair (BEAR) procedure: An early feasibility cohort study. Orthopaedic Journal of Sports Medicine. 21 Nov 2016;**4**(11):2325967116672176. DOI: 10.1177/2325967116672176

[46] Nepple JJ, Block AM, Eisenberg MT, Palumbo NE, Wright RW. Meniscal repair outcomes at greater than 5 years: A systematic review and meta-analysis. The Journal of Bone and Joint Surgery. American Volume. 2022;**104**(14):1311-1320 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/35856932/

[47] DePhillipo NN, LaPrade RF, Zaffagnini S, Mouton C, Seil R, Beaufils P. The future of meniscus science: International expert consensus. Journal of Experimental Orthopaedics. 31 Mar 2021;**8**(1):24. DOI: 10.1186/s40634-021-00345-y

[48] Kopf S, Beaufils P, Hirschmann MT, Rotigliano N, Ollivier M, Pereira H, et al. Management of traumatic meniscus tears: The 2019 ESSKA meniscus consensus. Knee Surgery, Sports Traumatology, Arthroscopy. 2020;**28**(4):1177-1194 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/32052121/

[49] Kaminski R, Kulinski K, Kozar-Kaminska K, Wielgus M,

Langner M, Wasko MK, et al. A prospective, randomized, double-blind, parallel-group, placebo-controlled study evaluating meniscal healing, clinical outcomes, and safety in patients undergoing meniscal repair of unstable, complete vertical meniscal tears (bucket handle) augmented with platelet-rich plasma. BioMed Research International. 11 Mar 2018;**2018**:9315815. DOI: 10.1155/2018/9315815

[50] Everhart JS, Cavendish PA, Eikenberry A, Magnussen RA, Kaeding CC, Flanigan DC. Platelet-rich plasma reduces failure risk for isolated meniscal repairs but provides No benefit for meniscal repairs with anterior cruciate ligament reconstruction. The American Journal of Sports Medicine. 2019;**47**(8):1789-1796 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/31166699/

[51] Kaminski R, Kulinski K, Kozar-Kaminska K, Wasko MK, Langner M, Pomianowski S. Repair augmentation of unstable, complete vertical meniscal tears with bone marrow venting procedure: A prospective, randomized, double-blind, parallel-group, placebo-controlled study. Arthroscopy. 2019;35(5):1500-1508.e1. [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/30902532/

[52] Piontek T, Ciemniewska-Gorzela K, Naczk J, Jakob R, Szulc A, Grygorowicz M, et al. Complex meniscus tears treated with collagen matrix wrapping and bone marrow blood injection: A 2-year clinical follow-up. Cartilage. 2016;**7**(2):123-139 [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/27047635/

[53] Ciemniewska-Gorzela K, Bąkowski P, Naczk J, Jakob R, Piontek T. Complex meniscus tears treated with collagen matrix wrapping and bone marrow blood injection: Clinical effectiveness and survivorship after a minimum of 5 years' follow-up. Cartilage. 2021;**13**(1_suppl):228S-238S [Internet] [cited 2023 Mar 5] Available from: https://pubmed.ncbi.nlm.nih.gov/32476440/

[54] Chahla J, Stone J, Mandelbaum BR. How to manage cartilage injuries? Arthroscopy - Journal of Arthroscopic and Related Surgery. 2019;**35**(10):2771-2773

[55] Gobbi A, Karnatzikos G, Kumar A. Long-term results after microfracture treatment for full-thickness knee chondral lesions in athletes. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;**22**(9):1986-1996

[56] Steadman JR, Rodkey WG, Briggs KK. Microfracture: Its History and Experience of the Developing Surgeon. Cartilage. 2010;**1**(2):78-86. DOI: 10.1177/1947603510365533

[57] Gobbi A, Lane JG, Dallo I. Editorial commentary: Cartilage restoration—What is currently available? Arthroscopy - Journal of Arthroscopic and Related Surgery. 2020;**36**(6):1625-1628

[58] Hoemann CD, Gosselin Y, Chen H, Sun J, Hurtig MB, Carli A, et al. Characterization of initial microfracture defects in human condyles. The Journal of Knee Surgery. 2013;**26**(5):347-355

[59] Gobbi A, Herman K, Grabowski R, Szwedowski D. Primary anterior cruciate ligament repair with hyaluronic scaffold and autogenous bone marrow aspirate augmentation in adolescents with open Physes. Arthroscopy Techniques. 2019;**8**(12):e1561-e1568

[60] Mithoefer K, Mcadams T, Williams RJ, Kreuz PC,

Mandelbaum BR, et al. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: An evidence-based systematic analysis. American Journal of Sports Medicine. Oct 2009;**37**(10):2053-2063. DOI: 10.1177/0363546508328414

[61] Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture. American Journal of Sports Medicine. 2016;**44**(11):2846-2854

[62] Gobbi A, Karnatzikos G, Kumar A. Long-term results after microfracture treatment for full-thickness knee chondral lesions in athletes. Knee Surgery, Sports Traumatology, Arthroscopy. 2014;**22**(9):1986-1996

[63] Frank RM, Cotter EJ. Nassar I. Failure of Bone Marrow Stimulation Techniques: Cole B; 2017

[64] Behery O, Siston RA, Harris JD, Flanigan DC. Treatment of cartilage defects of the knee: Expanding on the existing algorithm. Clinical Journal of Sport Medicine. 2014;**24**:21-30

[65] Gille J, Behrens P, Volpi P, De Girolamo L, Reiss E, Zoch W, et al. Outcome of autologous matrix induced Chondrogenesis (AMIC) in cartilage knee surgery: Data of the AMIC registry. Archives of Orthopaedic and Trauma Surgery. 2013;**133**(1):87-93

[66] Salzmann GM, Baumann GA, Preiss S. Spontaneous minced cartilage procedure for unexpectedly large femoral condyle surface defect. Case Reports in Orthopedics. 2016;**2016**:1-3

[67] Bonasia DE, Marmotti A, Rosso F, Collo G, Rossi R. Use of chondral fragments for one stage cartilage repair: A systematic review. World Journal of Orthopedics. 2015;**6**(11):1006

[68] Bonasia DE, Marmotti A, Mattia S, Cosentino A, Spolaore S, Governale G, et al. The degree of chondral fragmentation affects extracellular matrix production in cartilage autograft implantation: An In vitro study. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2015;**31**(12):2335-2341

[69] Stone KR, Pelsis JR, Na K, Walgenbach AW, Turek TJ. Articular cartilage paste graft for severe osteochondral lesions of the knee: A 10- to 23-year follow-up study. Knee Surgery, Sports Traumatology, Arthroscopy. 2017;**25**(12):3824-3833

[70] Hangody L, Dobos J, Balo E, Panics G, Rudolf Hangody L, Berkes I. Clinical experiences with autologous osteochondral mosaicplasty in an athletic population: A 17-year prospective multicenter study. The American Journal of Sports Medicine. 2010;**38**(6):1125-1133

[71] Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. The New England Journal of Medicine. 1994;**331**(14):889-895

[72] Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: A long-term follow-up. American Journal of Sports Medicine. 2010;**38**(6):1117-1124

[73] Niemeyer P, Salzmann G, Feucht M, Pestka J, Porichis S, Ogon P, et al. First-generation versus second-generation autologous chondrocyte implantation for treatment of cartilage defects of the knee: A matched-pair analysis on long-term clinical outcome. International Orthopaedics. 2014;**38**(10):2065-2070

[74] McCarthy HS, Roberts S. A histological comparison of the repair tissue formed when using either Chondrogide(®) or periosteum during autologous chondrocyte implantation. Osteoarthritis and Cartilage. 2013;**21**(12):2048-2057

[75] Mistry H, Connock M, Pink J, Shyangdan D, Clar C, Royle P, et al. Autologous chondrocyte implantation in the knee: Systematic review and economic evaluation. Health Technology Assessment. 2017;**21**(6):5-160

[76] Sadlik B, Gobbi A, Puszkarz M, Klon W, Whyte GP. Biologic inlay Osteochondral reconstruction: Arthroscopic one-step Osteochondral lesion repair in the knee using Morselized bone grafting and hyaluronic acid-based scaffold embedded with bone marrow. Aspirate Concentrate. 2017;**6**(2):e383-e389

[77] Gobbi A, Alvarez R, Irlandini E, Dallo I. Current concepts in subchondral bone pathology. In: Gobbi A, Lane JG, Longo UG, Dallo I, editors. Joint Function Preservation. Cham: Springer; 2022. DOI: 10.1007/978-3-030-82958-2_15

[78] Herman K, Whyte GP, Montagna A, Bizzoco L, Shabshin N, Szwedowski D, et al. Osteo-core plasty: Minimally invasive approach for subchondral pathologies. Journal of Cartilage & Joint Preservation. 2023;**3**(1):100101. DOI: 10.1016/J.JCJP.2023.100101

[79] Gobbi A, Dallo I. Osteo-Core-Plasty Technique for the Treatment of a Proximal Tibial Subchondral Cystic Lesion. A Case report of non-published data. Aspire Innovations June 2021. Available from: https://aspire-medical.eu/wp-content/uploads/2021/06/Osteo-Core-Plasty_CaseReport_Gobbi-Dallo_Jun21.pdf

[80] Szwedowski D, Dallo I, Irlandini E, Gobbi A. Osteo-core Plasty: A minimally invasive approach for subchondral bone marrow lesions of the knee. Arthroscopy Techniques. 20 Nov 2020;**9**(11):e1773-e1777. DOI: 10.1016/j.eats.2020.07.023