Recent Advances in Cartilage Repair (ICL 3)

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3.1 Introduction

Articular cartilage possesses low intrinsic healing property due to its lack of vascularity and progenitor cells. Thus, damage to the hyaline cartilage may lead to a progressive degeneration of the joint and eventually to osteoarthritis (OA). In the last years, different surgical techniques have been introduced in the clinical practice to overcome this issue. Bone marrow stimulation, for example, is a widely known method to allow cell invasion from the bloodstream to the site of damage. However, the reparative tissue has different morphological and biomechanical properties when compared to the native cartilage. In particular, the newly formed fibrocartilage has a low amount of proteoglycans and a higher concentration of type I collagen. This different matrix composition leads to a decrease in the mechanical strength and to a poor integration of the reparative tissue with the native cartilage.

For these reasons, new techniques have been developed to enhance the regeneration of the hyaline cartilage. In this regard, the integration between basic science and tissue engineering has led to promising results both in animal models and in the clinical practice. In particular, the increased knowledge in stem cell therapy has

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allowed for the introduction of bone marrow or adipose-derived mesenchymal stem cells for cartilage repair. Moreover, the advances in tissue engineering contributed to the development of new scaffolds, which may be eventually associated with a cellular component. These constructs often combine a bony part with a cartilaginous component; in fact, the importance of the subchondral bone in cartilage repair has indeed progressively increased, as many lesions affect both the chondral surface and the underlying bone.

The aim of this chapter is to describe the most recent advances in cartilage repair. Thus, we will first present in details the currently used techniques of bone marrow stimulation; then, we will give a brief overview on cell therapy and on osteochondral tissue engineering. In this regard, we will also summarize the latest animal and human studies on cartilage repair.

Finally, we will comment on the importance of the conservative treatment and physical therapy for focal cartilage lesions.

3.2 State-of-the-Art Treatment

3.2.1 Bone Marrow Stimulation

Marrow stimulation techniques are key first-line treatment options for small symptomatic articular cartilage defects [16]. Their guiding principle is to establish a communication of the articular cartilage defect with the subchondral bone marrow compartment. This is achieved (often arthroscopically) either by focal perforation of the subchondral bone plate with drill bits (subchondral drilling), awls (microfracture), or by its generalized and limited abrasion with round burrs (abrasion arthroplasty).

In general, marrow stimulation techniques are indicated for symptomatic small (<3–4 cm^2) focal chondral defects in young patients. Other indications are degenerative focal cartilage lesions with intact adjacent articular cartilage in middle-aged patients. Cartilage defects in juvenile patients are also another indication. Here, marrow stimulation is a first-line treatment option even for the larger defects (which might be treated by autologous chondrocyte implantation in adults). In elderly patients, marrow stimulation techniques are only rarely indicated.

Cartilage defect needs to be meticulously prepared. The borders of the defects are debrided to achieve stable and vertically oriented peripheral margins. The next step is the preparation of the cartilage defect base. The entire calcified cartilage layer has to be removed [6]. Then, marrow stimulation is performed either by subchondral drilling, microfracture, or abrasion arthroplasty. When the communication of the cartilage defect with the subchondral bone marrow compartment is established, a blood clot forms and pluripotent progenitor cells from the subchondral bone marrow subsequently migrate into the defect, differentiate into chondrocytes, and over time form a fibrocartilaginous repair tissue [40, 41].

A fibrocartilaginous repair tissue is the result of all marrow stimulation techniques. Good to excellent results have been reported in the majority of the cases. Physically active patients and patients younger than 30–40 years have better results. Also, the results are better when the defect is located in the femoral condyles, compared with the femoro-patellar joint [40, 41].

Hereafter, the individual techniques (subchondral drilling, microfracture, and abrasion) will be discussed and placed into perspective with data originating from recent translational animal studies.

3.2.1.1 Subchondral Drilling

Subchondral drilling was proposed for the treatment of osteochondritis dissecans (OD) by Smillie already in 1957 [53] and for osteoarthritis (OA) by Dr. Kenneth Pridie in 1959 [50]. Subchondral drilling is often termed Pridie drilling. When performing subchondral drilling, the tip of a Kirschner wire (K-wire) or drill bit is placed on the base of the prepared cartilage defect. At high speed, the rotating drill bit cuts through the subchondral bone plate into the subarticular spongiosa [47, 56]. Multiple drill holes are introduced into the subchondral bone plate of the defect, their numbers depending on the defect area.

Interestingly, Pridie recommended using a drill bit with a diameter of 1/4 in. (6.35 mm) in

his original publication. Nowadays, smaller instruments are more commonly used. In a rabbit model, Marchand et al. did not observe a specific effect of hole diameter on cartilage repair when two different drill hole sizes were applied to one single full-thickness cartilage defect in the trochlea [37]. On the other hand, larger holes would allow for an amplified access of reparative elements to the cartilage defect; however, they would induce a greater disturbance of the microarchitecture of the subchondral bone, while smaller holes might limit such subchondral bone damage by better reflecting the physiological subarticular trabecular distance. These two different opinions on hole diameter were tested in a sheep model of a full-thickness defect treated by subchondral drilling. After 6 months in vivo, drilling with 1.0 mm K-wire led to significantly improved histological matrix staining, cellular morphology, subchondral bone reconstitution, and average total histological score as well as significantly higher immunoreactivity to type II collagen and reduced immunoreactivity to type I collagen in the cartilaginous repair tissue compared with 1.8 mm defects. Moreover, restoration of the microstructure of the subchondral bone plate below the chondral defects was significantly improved after 1.0 mm compared to 1.8 mm drilling. Taken together, the data show that small subchondral drill holes that reflect the physiological trabecular distance improve osteochondral repair in a translational model more effectively than larger drill holes. These results have important implications for the use of subchondral drilling for marrow stimulation, as they support the use of small diameter bone cutting devices [7].

3.2.1.2 Microfracture

Microfracture was first described by Dr. John Richard Steadman about 20 years ago [55]. Here, multiple perforations of the subchondral bone plate are induced [55] with the sharp tip of a microfracture awl, allowing for the access of reparative pluripotent progenitor cells from the subchondral bone marrow cavity to the cartilage lesion [52]. Utmost care has to be taken not to penetrate the subarticular spongiosa too deeply or to damage the subchondral bone plate by a deflection of the cutting tip of the instrument [40, 42]. To avoid collapse of subchondral bone bridges created during the microfractures, it is advisable to start to perform the perforations for the lesion area close to the arthroscopic portal and then proceed onward, to avoid possible confluence of holes. Bone debris is carefully removed. Following the decrease of the arthroscopic pump pressure to about 30 mmHg, fat droplets and blood appear, confirming the successful performance of the marrow stimulation.

In a translational animal model, the hypothesis to test was that osteochondral repair is improved when the subchondral bone is perforated with small awls [46]. Full-thickness chondral defects in the knee joint of sheep that were debrided down to the level of the subchondral bone were treated with awls of two different diameters in a standardized fashion. Compared with untreated control defects, histological cartilage repair at 6 months was always improved following application of both awl sizes. Application of 1.0 mm microfracture awls led to a significantly improved histological overall repair tissue quality and surface when compared with larger awls. Subchondral bone cysts and intralesional osteophytes were frequently observed following either microfracture treatment [46]. The data show that small diameter microfracture awls improve articular cartilage repair in the translational sheep model more effectively than larger awls. From a clinical standpoint, the data support the use of small microfracture instruments and warrant prolonged clinical investigations.

3.2.1.3 Abrasion

Arthroscopic abrasion arthroplasty is a technique that has been described by Dr. Lanny L. Johnson in the 1980s. It is a modification of open Magnusson "housecleaning" arthroplasty [22]. Here, the subchondral bone plate of the defect is abraded – thinned out – by removing about 1.0–1.5 mm of its thickness, without completely eliminating the subchondral bone plate. It is thus different from a simple debridement, which is characterized by a sole removal of superficial cartilage fragments. The abrasion exposes the

vascularity of the subchondral bone plate, providing the connecting link to the subchondral bone marrow.

A rabbit study by Menche et al. investigated articular cartilage repair of full-thickness defects treated with abrasion arthroplasty versus subchondral drilling [39]. Animals treated with subchondral drilling had increased fibrocartilaginous repair, with a slight increase in degenerative changes. Abrasion arthroplasty produced a significant decrease in cartilaginous coverage of the exposed surface as well as progressive increase in degenerative changes [39]. A retrospective analysis of the clinical results of patients with isolated chondral lesions of the medial femoral condyle that were treated with arthroscopic abrasion showed at 10 years postoperatively and at final long-term follow-up at a mean of 20 years a positive functional outcome in 68 % of the patients [57]. In the same study, functional results for patients with small defects (<4 cm^2 area) were better than those for patients with large lesions. Abrasion arthroplasty has no proven value in the treatment of large osteoarthritic lesions.

Altogether, marrow stimulation techniques are important techniques indicated for small symptomatic lesions. They are technically feasible in most knee joint regions. Crucial technical aspects have to be respected. Marrow stimulation techniques are characterized by good clinical outcome within the first years postoperatively. Continuing clinical and translational research will further improve cartilage repair based on marrow stimulation.

3.2.2 Bone Marrow-Derived Mesenchymal Stem Cells for Cartilage Repair

In the last years, mesenchymal stem cells (MSC) have been presented as a valid alternative for the OA treatment (Fig. 3.1). The capacity to differentiate into cells of the chondrogenic lineage and produce extracellular matrix together with their proven anti-inflammatory potential brought to focus MSC as a potential treatment for OA.

Fig. 3.1 MSC preparation. Isolation of mesenchymal stem cells

MSC effects in chondrogenic repair have been documented in mice, rabbits, pigs, sheep, and horses. Francesc Soler's group published a feasibility and safety study in horse and ovine models, with intra-articular infusion of 40×10^6 autologous expanded bone marrow MSCs (BM-MSC), with no local or systemic pathological alterations seen in necropsy after 6 months, and showing clear chondral regenerative findings.

MSC may be obtained from bone marrow, adipose tissue, blood, periosteum, synovium, skeletal muscle, placenta, and deciduous teeth. But not all MSC offer the same versatility and therapeutic potential: the chondrogenic potential of BM-MSC "in vitro" is higher than those MSC from adipose tissue (AT-MSC). Some studies in animal model showed BM-MSC to be more effective than AT-MSC. BM-MSC generates cartilage lineage cells when cultured in TGF-β-enriched medium [34]. This should be considered when attempting to regenerate articular cartilage.

These encouraging results in animal model allowed to try translating the procedure to human therapy. Francesc Soler's group published the outcomes of a pilot study for knee OA treated with autologous expanded MSC (EudraCT 2009-017407-11 and NCT01183728). Twelve patients were treated by means of intra-articular infusion of 40×10^6 autologous expanded BM-MSC. Excellent results were reported according to pain (VAS), algofunctional, and

disability tests (Lequesne and WOMAC). No adverse side effects were described [45].

Cell therapy effectiveness is dose dependent: in human adults, MSC rate in bone marrow is 1:10.000–100.000 mononucleated cells (MNC), in G0 phase. Research on stem cell transplantation suggests that the clinical results depend on the dose [49]. Applying the product before expansion would render a low amount of MSC in G0 phase, not enough to expect some kind of effect in cartilage.

The main target of this therapeutic approach is OA, which is a diffuse deterioration of different joint areas, not a focal injury. In order to accurately evaluate the cartilage quality without performing a biopsy, Francesc Soler's group chose the T2 mapping MRI as a technique to determine the grade of disorganization of the extracellular matrix. In the pilot study, statistically significant changes in cartilage quality, assessed by means of T2 mapping MRI, were observed [45].

The same group proved the viability, security, and efficacy of the application of 40×10^6 autologous expanded BM-MSC for the treatment of knee OA under a single articular infusion. This study allowed to carry on with the treatment under the supervision of the Spanish Medicines Agency (AEMPS).

Recently Francesc Soler's group published the results at 12 months of the first 50 patients following the same procedure described in the pilot study [54]. All patients were satisfied with the treatment, and 43 out of 50 patients (86 %) reported lasting pain relief greater than 45 % throughout a 1-year observation period.

New studies assessing different cell doses and carriers to enhance cell viability and efficacy are indeed necessary, but in the meantime, the researchers concluded that there is a belief that treatment with autologous expanded MSC through infusion is a feasible, safe, and effective treatment for joint OA.

3.2.3 Lipoaspirate Injections for the Treatment of Early OA

As we mentioned earlier in this chapter, another feasible source of mesenchymal stem cells is the adipose tissue, which is indeed readily accessible and simple to harvest, and can be used to provide cushioning and filling of structural defects. In addition, adipose tissue has been shown to have an abundance of bioactive elements with phenotypic and gene expression profile similar to MSC and pericytes. These cells have been shown to secrete multiple trophic mediators, which act in a paracrine fashion within the recipient tissue to elicit angiogenic, antiapoptotic, and antifibrotic responses. Adipose-derived MSC is routinely obtained from the enzymatic digestion of fat lipoaspirates as stromal vascular fraction (SVF), which may undergo prolonged ex vivo expansion, with significant senescence and decline in multipotency. These techniques have complex regulatory issues, and they often lead to clinical results below expectations. We here present the efficacy and potential benefits of using minimally manipulated, autologous micro-fragmented adipose tissue (Lipogems®) in patients with knee OA. Compared to the enzymatically digested lipoaspirates, the Lipogems® product is composed with a significantly higher percentage of mature pericytes and MSC and lower amount of hematopoietic elements.

Lipogems® is a disposable device that progressively reduces the size of adipose tissue clusters, washing the tissue from pro-inflammatory blood, oil, and cellular debris through an "enzyme-free" minimal manipulation in an aseptic closed system, while maintaining intact stromal vascular niches with mesenchymal stem cells and pericytes (Fig. 3.2). The entire process is a one-step procedure, and it is performed in immersion in a saline solution, which minimizes any trauma to the cellular products.

The study included patients with knee OA. In all patients, the presence of OA symptoms was confirmed by clinical examination, X-ray, and MRI. Patients underwent a three-step procedure of lipoaspiration, adipose tissue processing using the Lipogems® device, and reinjection into the knee. Clinical outcomes were assessed using KOOS, KSS, and VAS pain scale and taken at baseline 1-, 3-, 6-, and 12-month follow-up.

The improvement of the symptoms occurred few days after treatment and steadily increased

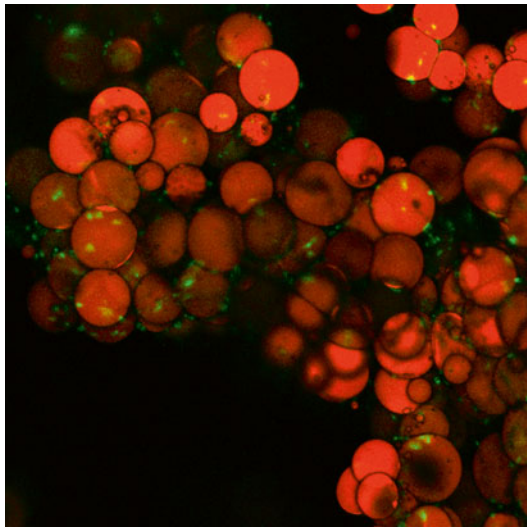

Fig. 3.2 Lipoaspirate. Microscopic image of the Lipogems® products

throughout the whole period of our study. The results of all KOOS subscales showed gradual statistically significant improvement of an average of 21.8 points for each subscale.

These results are very encouraging and point to Lipogems® as an easy, safe, and effective intraoperative procedure to obtain micro-fragmented minimally manipulated autologous adipose tissue for the treatment of knee OA.

3.2.4 The Use of PRP for Cartilage Lesions

Progresses have also been obtained in the use of platelet-rich plasma (PRP), which nowadays represents a valid and less invasive alternative to other bone marrow stimulation techniques.

PRP is indeed a blood derivative with a higher platelet concentration than whole blood. Platelets, once activated, release a group of biologically active proteins that bind to the transmembrane receptors of their target cells, leading to the expression of gene sequences, which ultimately promote cellular recruitment, growth, morphogenesis, and also modulate inflammation [14]. Thus, PRP represents an appealing biological approach to favor healing of tissues otherwise doomed by a low regenerative potential, such as cartilage. This led to the wide use of PRP in the clinical practice, showing promising results for the minimally invasive injective treatment of cartilage degeneration and OA. Therefore, an increasing number of both preclinical and clinical studies on PRP were performed and they overall displayed positive results [27].

Literature clearly demonstrates the safety of PRP injections, with no major adverse events recorded and only some reports of self-limiting immediate pain and swelling reaction [27]. Moreover, all studies seem to agree on an overall clinical benefit of PRP. Even recent randomized controlled trials (RCTs) have shown support in favor of PRP intra-articular injections, which have been shown to be better than saline injections, and some studies suggest a slight superiority of PRP with respect to viscosupplementation [17, 48, 51]. However, literature also presents some controversial findings, and the real potential of PRP for the treatment of knee degeneration is far from being proven. The largest available double-blind RCT comparing PRP and hyaluronic acid (HA) injections was not able to demonstrate any difference in the several subjective and objective outcome measures prospectively documented in 192 patients for up to 1-year follow-up [13]. Platelet concentration, dose, timing, and modality of application may have influenced the results, thus explaining the conflicting outcomes with other trials. It is also likely that many aspects such as cellularity, activation modality, mechanism of action, and targets need to be further explored to improve the potential of this biological treatment. It is also well known that the clinical benefit reported after PRP injection may be attributable to other action mechanisms. Both the rapid clinical benefit and the limited effect over time are in contrast with the timing required by an induced cartilage regeneration process. It is more likely that an intra-articular injection does not target only cartilage, as PRP might influence the entire joint environment. Some in vitro studies indeed confirm the effects of PRP on other cell sources

such as meniscal, synovial, and mesenchymal stem cells [14]. PRP might not lead to hyaline cartilage regeneration and might not change the clinical history with significant disease-modifying properties, but it still might offer a clinical and functional improvement and it might possibly delay the degenerative process. The clinical benefit is limited over time and can roughly be estimated in less than 1 year [27]; this outcome might suggest that this treatment should be applied in cycles to ensure longer-lasting results and postpone more invasive procedures.

Finally, another aspect emerges from the literature analysis. Not all patient categories present the same results, as younger patients affected by an early degeneration have a better outcome. Thus, it appears clear that there is room for a better targeting of PRP application. The understanding of the best treatment indications, together with the understanding of the mechanism of action of PRP will allow the optimization of the procedure and the improvement of this biological minimally invasive approach for the treatment of cartilage degeneration and OA.

3.2.5 Surgical Solutions for Osteochondral Defects

As mentioned in the introduction, the subchondral bone and its importance for a successful regenerative therapy of osteochondral lesions and the articular surface unit recently came into focus [15], as severe symptomatic and unstable osteochondral defects are difficult to treat [33]. Reasons for these lesions are, e.g., osteochondritis dissecans, osteonecrosis, or trauma. Traditional treatments for osteochondral defects consist of surgical transplantation of either autologous or allogeneic tissue. Autologous osteochondral transplantation was shown to offer a good and long-lasting clinical outcome [11], but with several limitations when addressing lesions bigger than 2.5 cm^2, due to donor site morbidity issues [12]. On the other hand, the use of allogeneic osteochondral plugs is a viable option for bigger lesions but presents limited availability. With the aim of overcoming the abovementioned limitations, regenerative strategies have been developed. Initially, techniques developed for the cartilage layer were modified to address osteochondral defects, such as ACI combined with the use of autologous bone to fill the bone defect [8]. However, a relatively high incidence of subchondral bone alterations has been highlighted for these procedures [47]. Moreover, high costs and morbidity, related to the double surgical procedure, pushed the development of new products with a bilayer structure reproducing the different biological and functional requirements of the entire osteochondral unit, in order to guide in one surgical step the growth of both bone and cartilage tissues, respectively [30]. The aim of these cell-free devices is to provide the right stimuli to regenerate the osteochondral tissue, supporting and guiding cell differentiation in situ toward bone and cartilage.

Among the many scaffolds commercialized for clinical application, a very few of them has currently been reported in the literature.

A bilayer scaffold made of a porous PLGA-calcium-sulfate biopolymer (TruFit, Smith & Nephew, Andover, MA) in form of mosaic-like cylinder plugs was the first reported. After promising preclinical results, the plug was initially introduced into the clinical practice for backfilling autologous graft donor sites, but it has also been directly implanted for the treatment of focal articular surface defects, where it showed some controversial findings [3, 62].

Dhollander et al. reported a failure rate of 20 % (3 out of 15 patients) at 12 months, paired with fibrous vascularized repair tissue at biopsies [5], and Joshi et al. reported 70 % of 10 patients undergoing a second surgical procedure due to implant failure within the first 24 months after plug implantation for patellar lesions [23]. Finally, the comparison with mosaicplasty in two groups of patients treated for similar defects showed significantly higher outcomes for the latter ones [20].

A three-layer nanostructured implant made of collagen and hydroxyapatite (MaioRegen™, Fin-Ceramica, Faenza, Italy), mimicking the

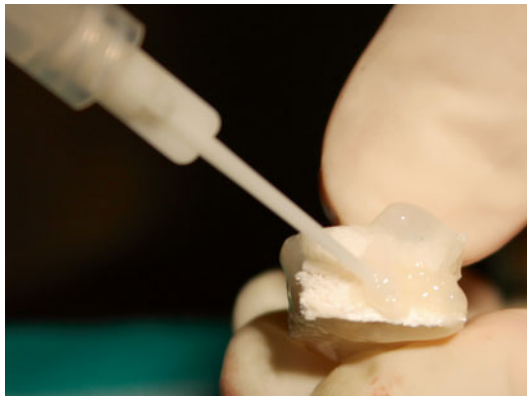

Fig. 3.3 Macroscopic picture of a Collagen-hydroxyapatite scaffold. The implantation technique involves the use of fibrin glue on the *top* and borders to maximize the primary stability of the patch

composition of the extracellular matrices of cartilage and bone tissue [59], showed promising results during in vitro and animal studies either with or without adding cells [25] and was therefore introduced in the clinical practice as a cell-free approach (Figs. 3.3 and 3.4).

Its clinical application has been widely reported up to midterm follow-up. A study on 27 patients showed a significant improvement in all the scores used that was stable until 60 months of follow-up. Also, MRI evaluation of 23 lesions revealed significant improvements in both mean magnetic resonance observation of cartilage repair tissue (MOCART) score and subchondral bone status over time. Nonetheless, some abnormalities persisted, even if no correlation was found between imaging and clinical outcomes [28].

Positive results at short-term follow-up have later been reported in a larger study on 79 patients [29], and the effectiveness of this approach was confirmed also in studies on specific patient subgroups, such as OCDs [9], tibial plateaus [32], large [2, 4], or complex [10] articular lesions involving the subchondral bone. Lastly, this biomimetic patch was successfully applied as part of a combined approach as salvage procedure for unicompartmental OA patients [36].

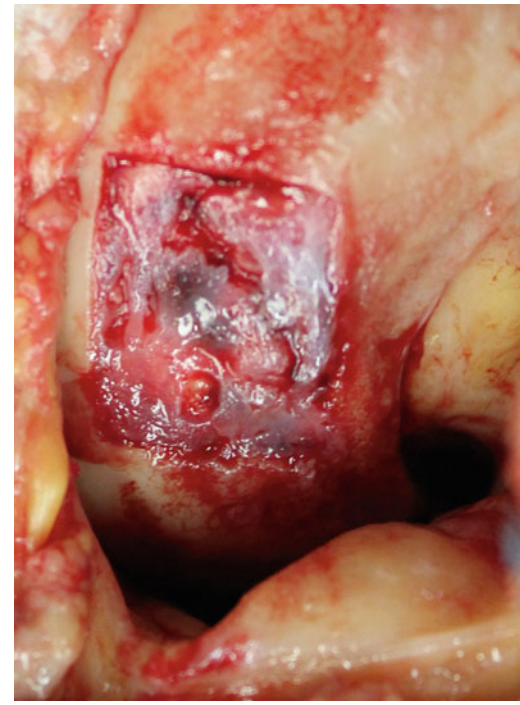

Fig. 3.4 Collagen – hydroxyapatite scaffold implantation for femoral condyle osteochondral defect. The articular surface and margins are covered with fibrin glue

More recently, an aragonite-based osteochondral scaffold was developed (Agili-C™, CartiHeal, 2009 Ltd, Israel). It is a rigid cell-free implant in cylinder shape that consists of two layers: a bone phase made of calcium carbonate in the aragonite crystalline form and a superficial cartilage phase composed of modified aragonite and hyaluronic acid. Preclinical analysis showed biodegradability and intrinsic restorative potential and the ability to recruit cells from the surrounding tissues, allowing the one-step implantation without any cell augmentation [31]. Currently, a single case report describing the clinical use of this construct is available in the literature: a 47-year-old nonprofessional sportsman affected by a post-traumatic osteochondral lesion around 2 cm^2 on the medial femoral condyle was treated successfully and resumed his pre-injury sport activity after 18 months. The MRI evaluation

performed at 24 months of follow-up also showed good results with the restoration of the articular surface, but larger studies need to be performed to confirm the promising preliminary findings [26].

3.2.6 Regenerative Treatment of Deep Osteochondral Defects

While many authors report good to excellent long-term results after treatment of small osteochondral lesion with osteochondral transplantation [19], less is known about treatment options for large and deep osteochondral defects, as the complication rate of osteochondral transplantation correlates to defect size. Few alternative treatment options are described in literature. However, resection of large adult OD lesions resulted in bad clinical outcome and development of OA. Refixation of large grade 4 ODs failed to integrate into the surrounding bone and showed no clinical improvement in long term [24]. However, in recent years, regenerative treatment approaches for large osteochondral defects showed promising results.

The combination of matrix-guided autologous chondrocyte transplantation (MACT) with bone augmentation has indeed been proposed [61, 65]. Ochs et al. saw a remodeling of articular cartilage and subchondral bone after bone grafting and MACT for treatment of deep OD lesions [44]. For bone augmentation monocortical cancellous cylinders were used to reconstruct the subchondral layer. The cartilage defect filling and the lamina remodeling grades correlated significantly with each other and clinical outcome. Vijayan et al. described a method of impaction bone grafting of the defect with cancellous bone harvested from the medial femoral condyle and covered with MACT [63]. However, some defect locations and geometries especially toward the notch border, where osteochondral defects are often located, are not suitable for impaction bone grafting due to the missing defect containment. Könst et al. used a full-thickness corticospongious autologous bone graft from the medial or lateral condyle for bone augmentation and covered it with a gel-type autologous chondrocyte implantation [33]. Although the reconstruction of the subchondral plate seems to be mandatory for a successful treatment of deep osteochondral defects [15, 44], there is still a lack of information about the best method to address the bony part of the osteochondral lesion.

In one of our more recent studies, we treated the largest number of patients with deep osteochondral defects with bone augmentation combined with MACT. According to defect depth and size, bone defect filling was performed with cancellous bone impaction or implantation of an autologous bicortical bone graft from the iliac crest covered with MACT. 51 patients were followed up at 3 and 6 months and 1, 2, and 3 years and clinically evaluated using the International Knee Documentation Committee (IKDC) score and the Cincinnati score. An MRI evaluation was performed at 3 months and 1, 2, and 3 years, and the MOCART score with specific subchondral bone parameters (bone regeneration, bone signal quality, osteophytes, sclerotic areas, and edema) were analyzed.

At the 1- and 3-year follow-ups, both the IKDC and the MOCART scores have significantly increased with the time. Thus, the new bone block augmentation technique combined with MACT might represent a valid treatment for large osteochondral defects.

3.2.7 The Role of Physical Therapy for Conservative Treatment

Despite the progressive improvement of techniques for cartilage repair, we should always remember that specific focal cartilaginous lesions can and should be treated conservatively, especially if young patients are involved. In these cases, physical therapy plays a major role in the conservative treatment. Thus, we dedicated a section of this chapter to the role of the physical

therapist in the rehabilitation of patients with cartilage lesions.

"The need for speed," "no pain, no gain," and "what doesn't kill you makes you stronger" intimidating myths? Yes, and the physical therapist (PT) should professionally deal with these myths.

Young athletes with knee cartilage lesions indeed present with clear mechanically induced articular and/or peri-articular complaints but with not well-recognized movement dysfunctions. When insidious cartilage injuries occur, the final diagnosis of underlying cartilage lesions takes time. Here there is a clear "need for speed." Frequently recurrent or persistent tendinitis or nonspecific joint line tenderness influences unfortunately to the great extent the power output and professional performance and puts the joint even in a vulnerable "prone to injury" position. Each PT should be able to recognize the clinical representations of cartilage injuries, the injury mechanisms, and the maladaptive or compensatory neuromuscular control strategies. Once the exact diagnosis of the cartilage lesion (size, location, concomitant lesions) is set, the "need for speed" simply applies on smart goal setting and criteria-based rehabilitation [43].

"No pain, no gain" and "fear avoidance" are possible behavioral movement strategies when confronted with pain. If patients behave continuously with one of these strategies, "undesirable and inevitable" pain will occur more easily, resulting in less capacity to enjoy physical efforts. Respectively, insight, respect, and renewed trust in healing and training should be restored or at least positively initiated. We "know" that local healing capacity of damaged cartilage is limited, one more reason to use a "feel good" approach with intense functional training.

"What doesn't kill you makes you stronger" does not take into account chondrocyte apoptosis. Chondrocytes are essential to maintain cartilage and its key functional characteristics of shock transducing and friction-free movement. Local mechanical overload and excessive shear forces during altered biomechanics can result in subclinical chondrocyte apoptosis. Since cartilage is aneural, surrounding innervated tissues such as the subchondral bone and the joint capsule inform us for possible threat. Typically, when clinical symptoms follow during joint reactivity or joint homeostasis loss, patients adapt their movement behavior.

The first goal of PTs is to inform patients and to help them to restore joint homeostasis. Exercise to facilitate neuromuscular control, temporary adjustments in activities of daily living (ADL), and intensifying training focus are typical to be addressed [64]. Specific low-load exercises can improve recovery of joint homeostasis, local nutrition state at the "repair" site, key signaling pathways to chondrocytes, periarticular lymphatic drainage, and local muscle tone and control [21]. Especially the local, more phasic muscles can dramatically loose muscle tone and need stimulation, preferably executed actively during ADL. Also in order to improve transfers with or without crutches, a temporarily adapted motor control strategy is recommended, of course depending on cartilage lesion site. If implemented correctly, the chances to locally overload the repair site, to provoke joint reactivity, and to increase pain perception are minimized. Besides neuromuscular retraining, proximal muscle strength exercises are desirable as soon as possible to overcome the "use it or lose it" phenomenon.

The role for physical therapy is both in analyzing movement strategies and follow-up training to improve joint function. Following cartilage injury, this is a lengthy process [60]. Fortunately, in the young athletes, good, satisfying progression is possible without jeopardizing a healthy fit future. Conservative treatment should be progressive but not aggressive. Following cartilage defects exclusive physical therapy may fail to restore full joint function. Consequently, and last but not least, an important role of the PT is to refer to a dedicated cartilage surgeon. The ideal timing of surgical cartilage repair interventions is not well documented. Some reports suggest an ideal window of opportunity between 10 weeks

and 6 months after cartilage injury. One should take this into account when no optimal functional recovery is reached with a progressive, criteria-based, conservative, and feel good treatment.

3.3 Future Perspectives

Preclinical and in vitro studies have recently suggested some intriguing glimpses in the future of cartilage repair.

Considering the continuing widespread use of scaffolds and matrices, some of the "seeds" of cartilage tissue engineering lay certainly in the development of a new generation of therapeutic tools that allow for a progressive release of growth factors able to promote chondrocyte differentiation and cartilage matrix production. These are generally called "smart scaffold" and are preloaded with different molecules as transforming growth factor-β (TGF-β), bone morphogenetic protein-2 (BMP-2), insulin-like growth factor-I (IGF-I), and others or even a combination of these factors. In this regard, recent in vitro experiences suggest that an alternative way to deliver growth factors may come from "viral infections." Actually, pre-made recombinant adeno-associated viral vectors, retroviruses, or plasmids carrying a gene for a bioactive protein as IGF-I, fibroblast growth factor-2 (FGF-2), growth and differentiation factor-5 (GDF5), TGF-β, or transcription factor SOX9 have been shown to increase the synthesis of cartilage matrix and to enhanced proliferation of both chondrocytes or MSCs. The combination of these viral vectors inside polymer scaffold or self-assembling peptides, which can form stable hydrogels, allows for an effective, progressive, and controlled delivery of genes to the cells. This "gene-activated matrix" is indeed conceived for a vector release controlled by scaffold degradation preventing passive bolus release of the gene, and they may reasonably represent a future perspective for cartilage repair. Obviously, when biotechnology meets engineering, new possibilities arise again, and one of the present options coming from this perspective is represented by the concept of nanostructured membranes. Nanoscaffolds, made by tridimensional texture close to the dimension of extracellular matrix components, allow for a better "cross talk" between cells and materials and are able to improve cartilage differentiation and matrix formation, but they offer also some biochemical advantages. Specifically, nanostructures (i.e., carbon nanotubes) are able to adsorb more growth factors than traditional scaffold components as collagen. Moreover, at the level of "nanospace," some interesting phenomena occur, and one can observe that MSC, in contact with membranes of electrospun fibers of poly-L-lactic acid (PLLA) loaded with nanoparticles of hydroxyapatite (HA), shows a chondrogenic differentiation pathway in the absence of any chondrogenic medium. So, all these first experiences are unique and fascinating and certainly, in the future, more can be expected from the science of biomaterials.

From the standpoint of the use of blood derivatives for cartilage repair, many aspects are still to be clarified following the recent conflicting evidences. Indeed, if the value of PRP alone as a chondrogenic device may be mistrusted, it is unquestionably accepted the strong potential of PRP as a natural well-tolerated and individualized pool of bioactive molecules. From this point of view, a combined use of PRP together with other biologic agents may be hypothesized as a potential therapeutic preparation to increase cartilage repair. Recent evidences have shown promising results of PRP associated with hyaluronic acid or vascular endothelial growth factor (VEGF) antagonist or TGF-β or granulocyte-colony stimulating factor (G-CSF). However, beside these captivating hypotheses, the continuing research for the proper method to obtain a preparation of PRP suitable for cartilage repair is still proceeding. At this regard, some new clues about the positive effect of monocytes and lymphocytes have been described, allowing for the definition of a neutrophil-depleted, mononuclear

cell-enriched (monocytes and lymphocytes) PRP able to promote collagen production as a putative formulation to be further studied for improving cartilage repair. Ultimately, the growing interest in platelets and their content has pointed out the importance of microvesicles and miRNA in platelet physiology and, recently, the delivery of miRNAs alone (i.e., miRNA 23b) has been used to promote chondrogenic differentiation of MSC. Future reports will reveal if this captivating paradigm may have a role as a therapeutic alternative for preclinical and clinical studies for improving cartilage regeneration.

Nevertheless, if all these elements may have an important role in cartilage tissue engineering, the key factor for cartilage repair is still the cell. Indeed, the choice of cell source is fundamental and recent clinical studies are offering multiple possibilities, from bone marrow concentrate or adipose stromal vascular fraction to autologous culture MSC derived from lipoaspirate or allogeneic MSC combined with chondrons, as presented in the recent IMPACT trial from Saris et al. [1]. Nevertheless, basic science lesson shows that new candidates are emerging in this horizon. Autologous or allogenic juvenile minced cartilage fragments may represent potential candidates of chondrocyte reservoir, considering the "activated" phenotype, observed in chondrocyte migrating from the "micro-explants," similar to the cell from the superficial zone of articular cartilage. Moreover, an appealing option may reside in the use of induced pluripotent stem (iPS) cells as an "immortalized non-tumorigenic cell line" to be differentiated toward chondrogenic pathway. As suggested by Takahashi et al. since 2007, iPS cells can be generated from adult human fibroblasts, differentiated into cell types of the three germ layers, and expanded infinitely [58]. So, iPS cell-derived chondrocytes may be obtained and applied in vitro and in vivo, even if a non-negligible risk of tumorigenesis (i.e., teratoma) has been observed in mouse models.

Moreover, a growing interest in the use of umbilical cord stroma (UC) as a source of stem cells is present in literature. Beside the well-known UC blood-derived mesenchymal stem cells (hUCB-MSC) [18], recent reports propose the use of cells derived from UC structure as a noncontroversial attractive alternative, since cells are derived from a formerly discarded material entangling few ethical problems and legal concerns. Indeed, the UC contains two umbilical arteries and one umbilical vein and a mucous proteoglycan-rich connective tissue, named Wharton's jelly, covered by amniotic epithelium. So, MSC can be isolated not only from mononuclear cell fractions of umbilical cord blood but also from umbilical vein subendothelial layer, from the outer layers of umbilical vessels (the perivascular region), from the intravascular connective space, and from the subamnion region. Furthermore, the cord blood seems to contain small amount of mesenchymal precursor cells and its efficiency is hampered by the low quantity of blood obtainable and a low success rate of isolation. Data from literature suggest that the frequency of circulating MSCs in cord blood is approximately 0.002 ± 0.004 per 10^6 initially plated cells, while the number of CFU-F from a "classical" stem cell source as the bone marrow can be estimated as 83 ± 61 per 10^6 [35]. Conversely, in our experience, from the UC obtained during cesarean birth, a mean of 32 g of UC can be retrieved [38] and, for each gram of original UC tissue, 0.8×10^6 cells are obtained. This "mixed" heterogeneous MSC population has been able to differentiate toward osteogenic, adipogenic, or chondrogenic pathway. Moreover, both in pellet culture and in tridimensional scaffold culture (namely, collagen I/III and HYAFf-11 hyaluronic acid derivative membrane), chondrogenic commitment of UC-MSC is enhanced in hypoxic environment (Fig. 3.5), similarly to that of bone marrow MSC. For all these reasons, we believe that UC-MSC may be an appealing potential source for clinical allogeneic use to treat chondral and osteochondral lesions, and they may well represent a candidate for "universal off-the-shelf" stem cell products in the field of orthopedic tissue engineering.

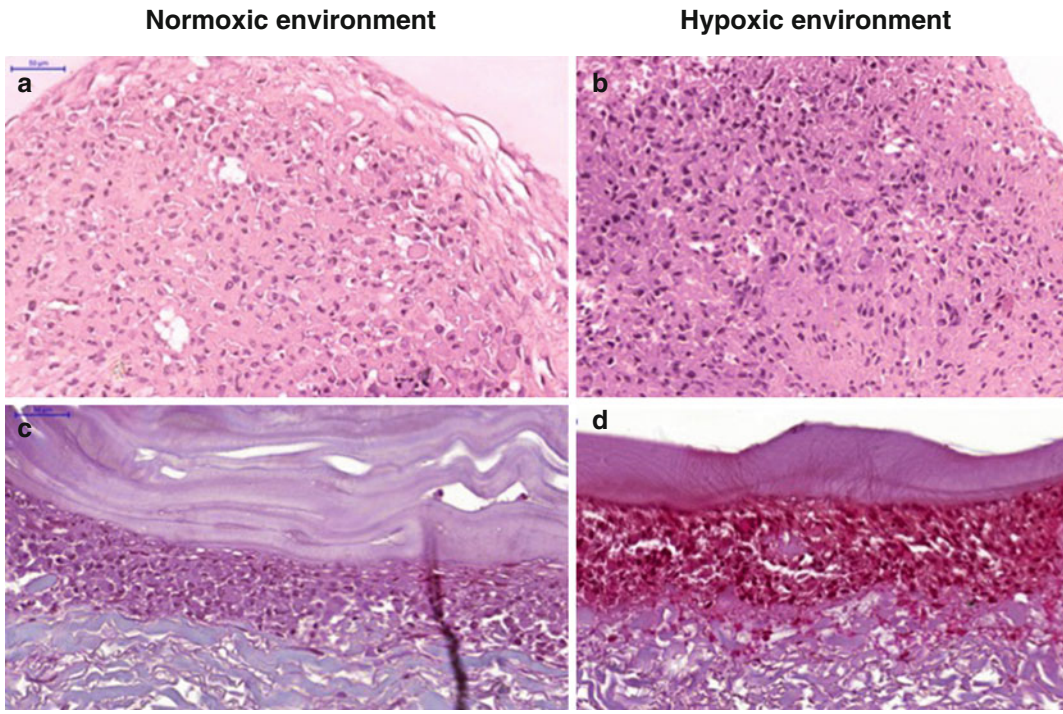

Fig. 3.5 Chondrogenic commitment of UC-MSC in hypoxic conditions. SAFRANIN-0 staining; (**a**, **b**) = pellet culture at 4 weeks, umbilical cord-derived mesenchymal stem cells (UC-MSC) at P2 were grown in chondrogenic medium; (**a**, **d**) = scaffold culture (collagen I/III) at 4 weeks, UC-MSC at P2 were stabilized at the top of the scaffold with fibrin glue and grown in chondrogenic medium; (**a**, **c**) = normoxic environment (21 % O_2); (**b**, **d**) = hypoxic (10 % O_2) environment; cultures grown at low oxygen tension showed more positive SAFRANIN-0 staining, consistent with increased sulfated glycosaminoglycan (*sGAG*) production, than that of cultures grown at standard normoxic conditions

Take Home Message

Cartilage repair still remains a challenge due to the specific properties of this tissue, mainly its avascularity and its lack of progenitor cells. Major improvements in this field have been made, thanks to the development of new tissue engineering techniques. In this chapter we described the most recent methods for cartilage repair. In particular, we focused on the novel strategies of cell therapy and on the new available biomaterials.

However, the choice of the best cell source and of the best biomaterial still remains a challenge; scientists are therefore trying to converge their efforts on these unsolved problems.

In conclusion, the future for cartilage tissue engineering so far appears an open landscape in which the combination of cells, membranes, and blood derivatives offers new fascinating pictures for cartilage repair. The best choice among all these strategies should take into account the type of damage, the general conditions of the joint, and also the patient's characteristics and expectations. Some of those treatments apparently seem still far from a clinical application; however, the "joint venture" of basic researchers and clinicians can shorten the distances, which are still too wide, because it is only this conjoined force that can shape the course of the future.

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