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Mesenchymal stem cells have for some time been gaining interest in the context of their potential applications in the treatment of musculoskeletal disorders. Mesenchymal stem cells (MSCs) are capable of differentiating into one of several mesenchymal phenotypes such as osteoblasts, chondrocytes, myocytes, marrow stromal cells, tendon–ligament fibroblasts and adipocytes [1–4]. Due to the relative ease of obtaining and administering them, compared to the alternative surgical treatment (or in combination with surgical treatment), they offer an attractive therapeutic option, for both physicians and patients alike, and find an increasing range of applications in orthopaedics. According to the most recent thinking on mesenchymal stem cell physiology, these cells are actually pericytes, that is, perivascular cells which are activated in response to trauma or local inflammation, and act to repair the damage using various types of chemotactic factors [5]. These secreted bioactive factors suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis and stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells [6]. It has been proposed that the pericyte is released from its position on a vascular tube in the case of a focal injury, and, as such, it functions as an immunomodulatory and trophic MSC [7]. MSC-induced immune modulation turns off T-cell supervision of the injured area and blocks autoimmunological reactions. Its trophic activity limits the field of damage so that scarring does not occur and that tissue-intrinsic progenitors replace the expired cells.

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In keeping with this understanding of the role of pericytes, it seems to follow logically that the therapeutic process could be based on such a natural, easily available source for body regeneration. To this end, cells could be harvested, condensed and administered in large quantities to the injured area, stimulating the surrounding tissue to heal.

At present, the use of stem cells in orthopaedics focuses particularly on the treatment of chondral defects (including those related to degenerative disease), meniscal injuries, and chronic tendinopathy and on accelerating healing of muscular injuries in athletes [8, 9]. A significant and most interesting part of research has gone into exploring the non-invasive, interventional applications of these cells.

Historically, the most often used source of MSCs has been the bone marrow. According to the new concept, whereby MSCs are pericytes found on capillaries, they can be harvested from practically any tissue. However, taking into account the ease of harvesting, minimal invasiveness as well as high cell concentration—adipose tissue seems to be the optimal source of MSCs. Studies have shown that 1 g of adipose tissue can yield approximately 5000 stem cells, which is 500 times more cells than can be obtained from an equivalent amount of bone marrow [10].

One important question to be asked in this context is whether the application of adipose-derived stem cells by injecting them into the target site affects the quality of the cells administered. In fact, no statistically significant differences have been found in terms of the quantity, viability and metabolic activity of the administered cells in comparison to control group, thus confirming that injection is a safe application method in cell therapy [11].

With regard to the impact of intra-articular administration of stem cells on the healing of articular cartilage damage, research studies have concentrated mainly on the knee joint [12–15]. In literature, we encounter two general methods of stem cell administration. Stem cells can be delivered directly into the joint without prior preparation of the damaged area (systemic degenerative disease), or such delivery may be preceded by the debridement of the focal chondral defect (arthroscopy), potentially with the additional application of tissue adhesive as sealant or carrier [16]. Yong-Gon Koh et al. [17] treated a group of patients aged over 65 by administering a single application of stem cells extracted from adipose tissue following arthroscopic debridement. Improvement was reported in all clinical outcomes (KOOS, VAS), the progression of lesions was found to be delayed in radiographic assessment according to the Kellgren–Lawrence scheme and the quality of cartilage was found to improve on second-look arthroscopy. Freitag et al. [18] have started a clinical trial aimed at analysing the impact of adipose-derived stem cell injections on focal defects in knee femoral condyles. To this end, microfractures were performed in the treatment group and the control group. The treatment group received an injection of mesenchymal stem cells following surgery. Preliminary findings are promising as statistically significant differences have been observed. An interesting solution was proposed by Yong-Gon Koh et al. [19], who biopsied the patients' infrapatellar (Hoffa's) fat pad to obtain adipose-derived stem cells identical to the place of subsequent application. Cells were harvested during standard arthroscopy. Isolated cells were then administered to the knee joint in combination with platelet-rich plasma. The clinical outcome was evaluated using the Lysholm, WOMAC and VAS scoring systems and significant improvement was noted across the board.

The use of stem cells in joint reconstruction surgery in order to accelerate implant fixation and healing is becoming increasingly popular in clinical practice.

Kosaka et al. [20] investigated the reconstruction of the anterior cruciate ligament in a rabbit model wherein the semitendinosus tendon graft was coated with fibrin glue enhanced with adipose-derived stem cells. Both rabbit groups included in the study underwent ACL reconstruction, but in the control group, the implant was coated with fibrin glue only. Comparison between the groups included the directivity of collagen fibres, graft tensile strength and the quality of Sharpey's fibres, which are signs of implant attachment. All of these parameters were higher in the group that had received adipose-derived stem cells. Kouroupis et al. [21] studied the

morphology and biomechanics of an implant developed with the use of biomedical engineering. In their study, they harvested human adipose-derived stem cells which they cultivated over 21 days on a biomaterial designed in such a way that a tendinous band would emerge between two ends induced to differentiate towards bone. Anterior cruciate ligament models created in this way were implanted in swine. The conclusions of the study confirm that the use of biomedical engineering and stem cell therapy in the treatment of knee ligament damage is the way to the future. In our opinion, this particular approach is quite costly, complex and time consuming. New techniques, which are more time and cost effective, using mechanical microfragmentation or fractionation of adipose tissue are presented as an alternative approach later in this chapter.

Chronic tendinopathy is a clinically relevant challenge, which frequently affects athletes. A study by Oshita et al. [22] was designed to take a closer look at the Achilles tendinopathy and its treatment using adipose-derived stem cell injections. In the injection group, significantly lower tendon degeneration was reported and a more favourable ratio of type III collagen to type I collagen.

In recent years, considerable effort has also gone into developing treatment methods for rotator cuff injuries with the use of stem cell injections into the affected area. However, no statistically significant differences have been reported. An interesting concept was put forward by Sevivas et al. [23] who investigated the impact on fatty infiltration of mesenchymal stem cell injections delivered into the site of chronic rotator cuff tendon injury. Researchers noted a statistically significant reduction of fatty degeneration and muscle atrophy in the rotator cuff, which may be of consequence for the decisions on undertaking surgical treatment.

One cannot fail to notice that practically all of the clinical reports on the application of stem cells in degenerative joint disease are characterised by a relatively short observation period and few are randomised. The test of time will provide a definitive answer regarding the efficacy of the above-mentioned therapies [24]. However, the positive 2-year results of therapy based on single injection of microfragmented adipose tissue in the treatment of early knee osteoarthritis, presented later in this chapter, may confirm the purposefulness of further investigation.

16.1 Lipoaspirate Grafting and Processing Methodology

The lipoaspirate is processed mechanically, according to technique described by Bianchi et al. [25], to eliminate blood and oil debris from fragmented adipose tissue, which could cause inflammation at the administration site. Additionally, special filters make sure that the final preparation is homogenous and can be applied with precision using even very small needles.

The device used consists of a transparent plastic cylinder with filters and beads for the microfragmentation of adipose tissue; tubes that guarantee the constant flow of saline solution and the elimination of waste products are connected to the cylinder. The system progressively reduces the size of adipose tissue clusters, washing out all of the pro-inflammatory oil and blood debris through an “enzyme-free” minimal manipulation in an aseptic closed system. The entire process, carried out in one surgical step, is performed in immersion in saline solution, which minimises any trauma to the cellular products.

The most common location for harvesting fat is the abdomen. In the general population, this would pose no problem, but in athletes the amount of subcutaneous adipose tissue is often very low. In such an event, the tissue may be harvested from alternative locations, for instance, the inner or outer thigh or the lateral sections of the trunk. In our experience, all of our lipoaspiration procedures (including those performed on exceptionally lean professional athletes) have been performed by the orthopaedic surgeon, but we may suggest that in the most challenging cases, a plastic surgeon could be asked to assist during the lipoaspiration. Before starting the procedure, it is advisable to examine the patient in a standing position to determine harvesting sites; seen as when lying down, the subcutaneous adipose tissue may be distributed in a significantly different manner and the body will usually look quite different. We also recommend that prior to starting, the planned lipoaspiration sites should be drawn on the skin with a marker.

During the procedure, the patient is lying in a supine position, if the fat is to be harvested from the front of the abdomen or thighs, or on their side, if the fat is to be collected from the (other) side of the trunk. A sterile field is prepared around

the aspiration site. While theoretically it is not necessary, the procedures in our centre are always performed in the operating room and in the presence of the anaesthesiologist, in case the patient requires additional sedation. The whole procedure takes about 20–30 min.

16.2 Subcutaneous Anaesthesia and Infiltration

A blunt-tip 19 G cannula connected to a 60 ml Luer Lock syringe is used to apply subcutaneously 500 mL normal saline, 50 mL lidocaine (2%), 1 mL epinephrine (1:1000)—about 50 ml per 10 cm^2 of skin surface. Wait approximately 15–20 min before proceeding with the harvesting of adipose tissue, to minimise potential bleeding and patient pain.

16.3 Lipoharvesting

Adipose tissue is harvested using a blunt-tip 13 G aspirating cannula with holes connected to a special Luer Lock syringe with progressive aspiration stops. The harvested fat is transferred to a larger syringe via a special connector. Once filled, syringes are stacked vertically so that fat can separate from water and blood. Excess liquids (blood and previously injected saline solution) are removed from the syringe.

16.4 Tissue Processing Set

The Lipogems set contains a connector to the saline bag, a container with steel marbles and filters where the adipose tissue is put in and then taken out after processing, and the wasting bag.

The device is first filled with saline to remove all air. This helps minimise cellular damage. Then the harvested aspirate is transferred into the device through a special filter, which breaks up the adipose tissue, and manually shaken for a minute or so. In the next step, the valves are opened and the lipoaspirate is washed in the saline, until clean, yellow-coloured preparation is obtained. The continuous flow of saline solution is intended to remove blood, oil and fibrotic tissue. The device should then be turned 180 degrees so that the tissue product can be pushed through the second filter, undergoing microfracturation of adipose clusters, into a syringe connected to the system via the Luer Lock. The clusters in thus obtained product are 300–600 mm large, so they can easily pass through a small needle. Syringes with the purified adipose tissue are placed vertically to decant, so that excess saline can be removed.

Fig. 16.1 Microfragmented adipose tissue

16.5 Injection

Final product—Microfragmented adipose tissue can then be administered directly into the joint using a syringe and needle (Fig. 16.1). About 6–10 ml of the preparation is administered to the knee area. In the case of smaller joints, the dose should be adjusted accordingly.

16.6 Results

Our study included a group of 20 patients with mean age of 61 years and OA of the knee grade 1, 2 and 3 according to the Kellgren–Lawrence classification.

In all the patients, the presence of osteoarthritis symptoms was confirmed by clinical examination, X-ray and MRI. Patients underwent a three-step procedure of lipoaspiration, adipose tissue processing and 6–10 mL reinjection into the knee through lateral suprapatellar approach. Results were assessed using KOOS questionnaire before the procedure (time zero) and then at 12 and 24 months' follow-up. X-rays and MRI were also collected.

Significant increase in KOOS scores—Ten-point increase was considered minimal important change (MIC).

The improvement of the symptoms occurred few days after treatment and increased steadily throughout the whole period of the study in KOOS and VAS pain scale. On VAS (visual analogue scale), the evaluation of pain decreased from severe to mild.

The complications we observed after the procedure were temporary and not significant. There were subcutaneous haematomas and tissue fibrosis. We did not observe fever or infections.

It is worth noting that the greatest improvement was reported on the KOOS Sport scale. We believe this may be due to the fact that patients willing to undergo such a procedure were actively seeking for alternative treatment methods that would enable them to regain physical fitness, which had been impaired by pain. Hence the conclusion that Lipogems offers effective pain relief enables people with early osteoarthritis to continue with sports activities. Here we also present an interesting observation of regeneration of cartilage and meniscus tissue on one of treated patients.

The patient was a 60-year-old man with osteonecrosis of medial femoral condyle and osteoarthritis of medial compartment. Thirty years before he underwent subtotal medial meniscectomy, he was treated with high tibial osteotomy about 3 years before, but he was still complaining about the pain and stiffness of the knee. The patient was treated with single injection of microfragmented adipose tissue. After 3 months KOOS scores significantly improved as well as VAS pain scale. As shown in the 3T MRI (Fig. 16.1), we observed healing of the bone of medial, femoral and tibial condyles. In addition, we observed covering of the medial femoral condyle with newly formed cartilage-like and meniscus-like tissue in place of the removed medial meniscus. We also observed less fluid in the knee, which corresponded with clinically significant less swelling and functional improvement (Fig. 16.2).

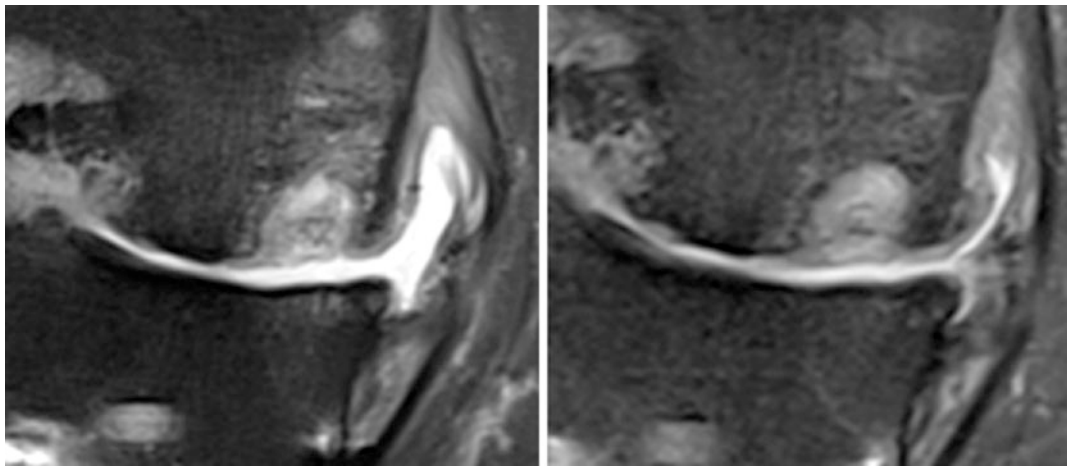

Fig. 16.2 (*Left*) 3T MRI before microfragmented adipose injection; (*right*) 3T MRI 3 months after microfragmented adipose treatment injection

16.7 Introducing Platelet-Rich Stroma for the Treatment of Osteoarthritis

As an alternative to the method described above, the recently introduced procedure of fractionation is presented to fabricate a more pure stromal vascular fraction [26]. The microfragmentation technique mentioned before is about crushing a certain, undefined part of the adipocytes present in the lipoaspirate, and as such it does hold ASCs and adipocytes and does provide repair by the former cells as described above.

The newly described technique of mechanical fractionation of nearly all adipocytes from condensed lipoaspirate results in a 10 percent volume of stromal vascular fraction. This tissue-SVF is almost completely free of adipocytes and has the theoretical advantage of a much higher concentration of ASCs in a smaller volume. Seven to eight times more cells are present in only 10 percent volume of the initial condensed lipoaspirate. A 20 cc of regular decanted lipoaspirate will yield about 10 cc of condensed lipoaspirate after a first round of centrifugation (9.5 cm radius fixed-angle rotor for 4 min, Medilite™, Thermo Fisher Scientific, NY). Subsequently 10 cc of condensed lipoaspirate is swooshed 30x times forward and back over a fractionator (Tulip, 1.4 mm hole Luer-to-Luer-transfer, reusable or disposable). Second round of centrifugation, 3 min 3000 rpm, will deliver four fractions: 85% oil (ruptured adipocytes), 10% of tissue-stromal vascular fraction (t-SVF), 5% aqueous fraction with a small pellet (of mostly single cell SVF, c-SVF). These fractions can be easily separated rendering 10 percent volume of SVF, ready for injection.

Compared to the microfragmentation method described above, less lipoaspirate has to be harvested, reducing donor site morbidity. At the same time, more ASCs remain for injection in a smaller volume, allowing for delivering higher number of ASCs in their natural niche into smaller spaces with almost no adipocytes. Also the attributes needed are less expensive.

The senior author of the paper (Stevens, HP, co-author of this chapter) is a plastic surgeon, originally developed the technique to improve regeneration of damaged skin (for the treatment of damaged skin due to ageing, radiation or (surgical) trauma, scars). Based on earlier findings demonstrating that platelet-rich plasma (PRP) could reduce postinjection downtime by 30%, both retrospectively [27] and in a prospectively randomised clinical trial (personal communication, to be published), about 50 cases of PRP + SVF were performed. This combination is referred to as platelet-rich stroma (PRS).

Not only a clear improvement of skin quality was observed for scar tissue [26], but also aged facial skin assessed by VISIA UHD computer photography showed improvement, and alopecia androgenica that was treated with PRS showed a significant increase of hair density (Stevens HP et al., personal communication, to be published).

Case report of treating knee osteoarthritis with PRS with 6 months follow-up, WOMAC score is presented (Stevens HP, et al., submitted for publication). A 62-year-old male, former professional soccer player, was treated with a single intra-articular injection of PRS (platelet-rich stroma; PRP; and FAT-SVF, fractionated adipose tissue-stromal vascular fraction) in his left knee. The degree of osteoarthritic joint destruction was severe (Kellgren–Lawrence grade 3) and a total knee replacement was scheduled already. The FAT-SVF was prepared as described above. PRP was prepared by using the autologous conditioned plasma (ACP) double-syringe system. The emulsified mixture of 4 cc of PRP and 1 cc of FAT-SVF was injected through a lateral suprapatellar approach under local anaesthesia within 45 min (Fig. 16.3). Prior injections with corticosteroids and hyaluronic acid did not result in any pain relief.

Postinjection effects were measured using an adapted Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Directly within the first 2 weeks postinjection, pain and stiffness reduced significantly. Physical, social and emotional functioning improved dramatically. No adverse effects were noted. During increased physical activities from 4 to 8 weeks postinjection,

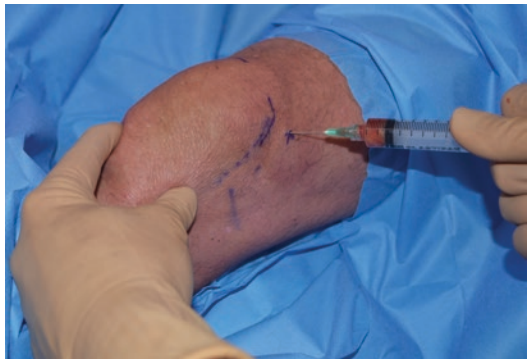

Fig. 16.3 Injection of platelet-rich stroma (PRS); the mixture of platelet-rich plasma (PRP) with stromal vascular fraction (SVF) prepared by fractionation of adipose tissue (FAT) procedure)

a mild relapse of discomfort occurred. After taking some rest for a week, all parameters improved again up to 12 weeks postoperatively. Almost completely free of pain, not using any painkillers for weeks now, instead of having a total knee replacement, he was able to accept an offer as assistant football coach (in a professional international soccer club playing Champions League football). In the weeks thereafter, the increased activities and attempt to play ball a little bit resulted in increased pain. Painkillers were used again for 4 to 6 weeks. Reduction of physical stress and exercise allowed recovery of WOMAC scores as depicted to less than 50% of the original pre-injection levels of pain. Ultimately a knee replacement procedure might still be necessary at some date, but the socio-economical and financial benefit of the treatment and being able to postpone such an intervention are obvious.

This new procedure treating tissue damage by platelet-rich plasma enriched adipose stromal vascular fraction, and osteoarthritis (of the knee) in particular does seem promising and deserves further attention to our opinion.

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