Chapter

The Regenerative Effect of Intra-Articular Injection of Autologous Fat Micro-Graft in Treatment of Chronic Knee Osteoarthritis

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Abstract

Osteoarthritis (OA) is one of the most prevalent conditions resulting to disability particularly in elderly population About 13% of women and 10% of men aged 60 years and older have symptomatic knee OA. The proportions of people affected with symptomatic knee OA is likely to increase due to the aging of the population and the rate of obesity or overweight in the general population. There are multiple factors associated with this progressive disease such as obesity, female gender, and repetitive trauma. Pain is the most common symptom in knee OA, a leading cause of chronic disability, clinical diagnosis will be supported by certain radiological findings. There are numerous conservative therapies that help to relive symptoms depend on severity of Osteoarthritis, and knee replacement remains standard of care in advance disease. Fat Micrografting is evolving technique with promising result in selected patients with regenerative and reparative effect of adipocyte-derived stem cell toward damaged cartilage and bone, which supported by clinical evidence.

Keywords: autologous fat micrograft, knee osteoarthritis, intra-articular injection, cartilage degeneration, adipocyte stem cells

1. Introduction

Rheumatic and musculoskeletal diseases (RMDs) constitute a group of more than 150 Conditions that are commonly characterized by progressive lesions and painful symptomatology. Altogether, they account for the leading cause of morbidity and disability worldwide, giving rise to tremendous health expenditures and professional incapacity. Osteoarthritis (OA), one of the RMDs, is a degenerative condition that principally involves the joint's cartilage, leading to its progressive destruction. It is related to aging and lifelong continual stress on the most functional articulations such as the knees, hips, and fingers, and the lower spine region. OA ranks among the ten most disabling conditions in developed countries. The global prevalence of symptomatic OA is estimated as 9.6% in males and 18.0% in

females over 60 years of age. Further, 80% of individuals with OA would experience significant movement limitations, and 25% would have serious handicap to perform routine activities of the daily life [1].

The prevalence of OA varies in different regions of the world, with rates ranging from 3.8–70%, depending on the methodology of studies, whether clinical, radiographic, patient self- reporting, or physician diagnosis [2]. As the incidence and prevalence of OA increase with age, the extending life expectancy results in a growing number of people afflicted with OA, with a proportional risk of disability. In the United Kingdom, 20–30% of the elderly population (aged 60 years and above) are diagnosed with symptomatic OA [3]. In the Middle East countries, including Iraq, Yemen, Saudi Arabia, and Syria, more than one million people are estimated to have OA [4]. Approximately 85% of individuals over the age of 75 experience some symptoms of OA [5].

Knee pain represents more than 80% of the total burden of OA [6]. High body mass Index (BMI) has become an epidemic in the US in recent decades and is a well-known risk factor for knee OA [7]. In Saudi Arabia, a clinically based epidemiological study, at a primary healthcare clinic, by Al-Shammari et al. showed a prevalence of OA as high as 57.2%. Other data by Al Arfaj estimated the prevalence of knee OA as 53.3% and 60.9% in males and females respectively [8, 9].

The treatment of knee OA may use conservative measures including medications, Physiotherapy, and local injections, or surgical approach including total knee arthroplasty (TKA). TKA is highly effective in reducing articular pain and is associated with acceptable functional outcomes. The procedure of TKA is safe and is considered one of the most common and successful procedures in orthopedics.

2. Risk factors

Blagojevicy et al. did a systemic review and meta-analysis to study the risk factors of Knee OA in older patients. They included 85 out of 2233 studies screened. The main risk factors found were obesity, previous trauma, hand OA, female gender and older age [10].

High body mass index (BMI) is associated with development of knee OA, and it was proven that the physical disability of the patients affected by knee OA reduced after weight reduction [11].

Another meta analysis conducted by Muthuri et al. showed that knee injury's history is one of the major risk factors associated with Knee OA and it should be included in any prevention program since it is preventable factor [12]. Genetic factor as shown in different studies is also associated with OA [13]. Increased loading or mal alignment of the joints is considered to be other factors that may lead to OA. So, in summary the risk factors can be classified into patient related factors like BMI, genetic, gender and age and joint related like previous injury, abnormal loading or malalignment [14].

3. Staging

In research, multiple variations of the Kellgren and Lawrence staging system have been used. However, the original one is: [15].

- grade 0 (none): definite absence of x-ray changes of osteoarthritis.
- grade 1 (doubtful): doubtful joint space narrowing and possible osteophytic lipping.

- grade 2 (minimal): definite osteophytes and possible joint space narrowing.
- grade 3 (moderate): moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends.
- grade 4 (severe): large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends.

4. Management

General and clinical assessment of the patient will help in determining the appropriate. Treatment of osteoarthritis. The assessment of OA effect on patient's function, daily activity, social relationship and quality of life should precede any treatment. The. Management plan has to be discussed with the patient thoroughly including the education of osteoarthritis, benefits and risks of various treatment options.

4.1 Non-surgical management

There are different non-surgical methods to treat joint OA. According to the Osteoarthritis Research society International (OARSI) guidelines published in 2019, patient education and land-based exercise with or without management of dietary weight are core treatments of knee related osteoarthritis. Non-steroid anti-inflammatory drugs (NSAIDs) are recommended in OA.

Management and its topical derivative are strongly recommended for patients with knee OA. COX-2 inhibitors or NSAIDs with proton pump inhibitor should be utilized in patients with gastrointestinal pathology. Oral NSAIDs are not recommended for patients with cardiovascular diseases. Intraarticular injection with corticosteroids or hyaluronic acid is mainly used for knee OA. It is not recommended for poly articular or even hip OA. All these non-surgical methods are associated with different level of evidence in the literature [16].

4.2 Surgical management

Before surgical intervention is considered, the patient should have received the core conservative treatment. Arthroscopic debridement and lavage are not routinely recommended unless there is a clear justification such as mechanical block that can be resolved arthroscopically [17]. After failure of all non-surgical treatments and in advanced joint arthritis, the total joint arthroplasty using artificial joint is the best option. It should be done before the patient gets advanced functional limitations. It is very safe procedure and associated with sound outcome. Pre- and post-operative patient engagement in terms of having proper education and well-structured physiotherapy is crucial to end up with great results. The American Academy of Orthopedic Surgeons (AAOS) in their 2nd edition evidence-based guideline for the treatment of knee OA has published 15 recommendations. This includes self-management exercise programs, weight reduction for painful knee and BMI < 25 and NSAIDs (oral or topical) or tramadol for symptomatic patients. They could not recommend the use of glucosamine and chondroitin, Intra articular Hyaluronic acid injection and arthroscopic lavage and or debridement for patient diagnosed with knee OA. They were unable to recommend for or against the using of knee corticosteroid, growth factor, platelet rich plasma (PRP) intraarticular injections and arthroscopic partial meniscectomy for a torn meniscus in patients with

symptomatic OA. They also have stated that high tibial vulgus osteotomy might be performed for patients with painful medial knee joint OA [18].

5. Fat graft evolution

Neuber presented history of fat transplantation in literature initially in 1893 and he stated that smaller fat parcels tend to undergo less absorption [19], followed by communication from Czerny [20] Lexer [21], and Rehn [22]. In 1911, Bruning was the first to transfer autologous fat into the subcutaneous tissue for the purpose of soft-tissue augmentation [23]. 1950 Peer published 1st book about fat grafting in 300 pages, mentioned that survival rate of fat graft could be 50% and determined the viability of fat tissue which injected [24], in 1980 s liposuction technique evolved which improved technically over time, 1985 Illouz [25] and Fournier [26] developed an comprehensive approach to fat transfer by syringe harvesting, called "microlipoinjection". Fat harvest from liposuction became the simplest and easiest method to pursue fat grafting, which indicated to treat soft tissue depression and contour deformity. 1990 Coleman described steps of fat injection procedure and coins the term Lipostructure [27].

There is numerous indications for fat graft in esthetic and regenerative medicine, Fat grafting technique evolved over period of time and become standard of care use in esthetic and reconstructive cases, in era of regenerative medicine, Lipoaspirate is consider source of fat micrograft which contains adipocyte derived stem cell (ADCS), growth factors, preadipocytes and cytokines demonstrate promising clinical application in wide spectrum of pathology to regenerate and reform damaged biological structure and improve outcome as in Osteoarthritis which consider leading cause to disability particularly in elderly population [28].

6. The use of microfat graft for cartilage repair

Cartilage contain a small number of cells known as chondrocytes, which are responsible for maintaining a large extracellular matrix, 85% of cartilage constitutes water and two categories of molecules: collagenous and noncollagenous [29], The main function of cartilage is to protect underlying bone from friction and act as gliding surface to enable motion, Articular cartilage is characterized as avascular, aneural, and alymphatic and, at maturity, of low metabolic activity [30], which entitle cartilage to special tissue with difficult task to repair it self, It has been demonstrated that early in the process of cartilage damage there is a rapid loss of glycosaminoglycans from the tissue [31]. Thus, most large defects fail to heal, leading to a long-term prognosis of osteoarthritis [32].

7. Surgical technique to harvest fat

Fat is readily available and simple to harvest, with the fat grafting surgery itself shows a low donor-site morbidity, and is inexpensive and repeatable. Liposuction is considered one of the most frequently performed surgical procedures all over the world, Since its introduction in 1982 using a blunt cannula attached to a suction generating device, the procedure has been improved [33]. Current technology for liposuction includes suction-assisted lipectomy, ultrasound-assisted, power-assisted, laser-assisted, and radiofrequency-assisted liposuction [34].

7.1 Harvesting of the adipose tissue

The first step in fat transfer to harvest fat by Many different liposuction techniques, all with the aim of minimizing adipocyte damage and increasing its survival Chosen Donor site is pretreated with a tumescent solution which containing an anesthetic mixture of Lidocaine, Sodium bicarbonate to overcome the acidity of mixture and to reduce pain and discomfort at injection site,epinephrine to control bleeding by vasoconstrictive action and Normal saline, proportionally mixed according to surgeon preference, volume to be injected determine by the desire volume of fat harvest and accordingly 2–3 cc of tumescent mixture to each 1 cc of anticipated fat harvest.

There are a variety of suction methods from which one may choose. The standard techniques are the manual (Coleman technique) or suction-assisted liposuction (SAL). Negative pressure is applied With the Coleman technique and SAL in combination with gentle forward and backward movement of the cannula, causing physical disruption, thus allowing fat tissue harvest. These methods represent the current gold standard, and reports show a lack of stem cell damage and preservation of their regenerative potential [35].

There are multiple modifications of this method, including power-assisted liposuction (PAL), water jet- assisted liposuction (WAL), laser-assisted liposuction (LAL), ultrasound-assisted liposuction (UAL), and VASER (Vibration Amplification of Sound Energy at Resonance).

They all are developed to further facilitate the process of suctioning, with minimal trauma to the donor site and maximal outcome in the requested esthetic result.

Large-bore cannulas decrease the mechanical sheer stress on the harvested cells, and subsequently increase the total number of viable aspirated cells, and Studies have showed an inverse relationship between cellular damage and the diameter of the instrument used to extract fat [36].

8. Processing of lipoaspirate

The goal of processing is to eliminate cellular debris, a cellular oil and excess of infiltrated solution [37]. These elements cause inflammation at the recipient site, which can be unfavorable for the fat graft. Also, blood must be removed as it accelerates the degradation of the transplanted fat.

Sedimentation: little traumatic and gives a large number of vital and intact adipocytes. However, this method contains smaller concentrations of stem cells and a substantial amount of cellular debris and thus making it harmful to graft survival [38].

Filtration techniques: more efficient in producing viable graft material for large-volume fat transfers. One example is Puregraft filtration system; which is a closed-membrane filtration system that was originally designed to prepare fat for isolation of the stromal vascular fraction. Another example of filtration is lipoaspirate filtered with cotton gauze; this results in concentrating the fat and separating it from the infiltrated solution, oil and cellular debris. This method, as compared to centrifugation, showed no significant differences in the viability of transplanted fat cells.

Washing with normal saline: this preserves mesenchymal stem cells as well as a great number of adipocytes.

Centrifugation: considered the most frequently used technique. It separates fat from substances that increase the degradation such as blood, proteases, lipids, lipases, and it may concentrate the adipose stem cell fraction, potentially enhancing

graft survival, Coleman suggested a processing method where centrifugation speed is 3000 rpm for 3 min. This creates multiple layers; the upper level is composed primarily of oil, the middle portion is fatty tissue, and the lowest portion, which is the densest layer, is composed of fluids and blood. This method obtains the highest possible concentration of stem cells within aspirate. It has also the increased content of angiogenic growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). To note that excessive centrifugal force may damage intact adipocytes and on the other hand better graft viability with low centrifugal forces.

9. Microfat graft preparation

Processing micro fat graft that require to process harvested fat graft through multiple micro pores filters that allow micro fat graft to pass through, these filters have different sizes that connected to Leur-leur 1–10 cc syringe.

10. Fat graft injection

Adipose tissue is injected in to the transplantation site through cannulas, where multiple tunnels are created on insertion, and fat is injected during withdrawal of the cannula. Graft through nutrition by tissue fluid absorption can survive up to 48 h. Meanwhile, neovascularization is being established. Therefore, the diameter of the graft should not be more than 2 mm to avoid central necrosis. The osteoarthritic knee joint was injected with autologous intra-articular fat micrograft 15–20 mL through the lateral approach according to the case in an amount that did not produce high pressure inside the joint and did not produce pain to the patients due to tension of the joint capsule [39].

11. Postoperative care

Postoperative care include antibiotics for one week, pain killers, and garment pressure dressing at injection and donor sites, encourage exercise and physiotherapy, massage to reduce swelling.

12. Complications

Infection is the most devastating encounter complication which presented as redness, hotness, increase pain, and purulent collection, treatment depend on severity of infection ranging from I.V antibiotics in addition to incision and drainage.

13. Fat is source of adipocyte derived stem cells (ADSC)

Adipose tissue composed of mature adipocytes (>90%) and a stromal vascular fraction (SVF), which includes preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, lymphocytes, ADSC, cytokines and growth factors [40–46], led to a growing interest for the use fat graft as regenerative therapy for common bone and joint diseases, with promising

therapeutic clinical application of ADSC into skeletal system with underlying structure such as Muscle, cartilage, ligament, tendon, and bone with regenerative and reparative potential and ADCs are considered as an ideal source of cell therapy for different types of diseases including bone and joint diseases [47].

14. Adipocyte derived stem cell role in regenerate knee osteoarthritis

Adult stem cells, represented mainly by the mesenchymal stem cells (MSCs) are present in most organs and tissues of the human body; they intend to replace damaged cells as a normal process [48]. These stem cells do not have ethical or legal concerns as compared to the embryonic and fetal stem cells [49]. Mesenchymal stem cells are a promising tool for tissue regeneration. They are multipotent adult stem cells obtained from different sources like bone marrow (BM), adipose tissue, umbilical cord, placenta, synovial membrane [50]. These cells because they are located in fat and synovial membrane, they are most suitable for the treatment of osteoarthritis (OA) [51].

The International Society of Cellular Therapy (ISCT) defines mSCs by three characteristics [52]. They need to be plastic adherent, express specific markers like the CD105, CD90, and negative for CD34, CD45, HLA-DR [47, 53, 54]. MSCs can differentiate to mesodermal lineage cells (osteocytes, adipocytes, chondrocytes) [54]. These cells differentiate also into many other cell types, like myocytes, neurons [55], cardiomyocytes and hepatocytes [56] in vitro and in vivo [57–59].

They are non-immunogenic cells as they lack the expression HLDR receptor, which makes them suitable for allogeneic transplantation [59]. These cells are capable of suppressing lymphocyte reactivity [60] and inhibit the production of inflammatory cytokines in vitro [61]. MSCs express cytokine and chemokine receptors on their cell surface, which allows them to migrate to the site of injury [62]. The ability to suppress the immune response enabled their use in graft-versus-host disease and transplant rejection [63].

Each of the MSCs depending on their origins presents some differences. The BM-MSCs have high differentiation capability, but are difficult to get from bone marrow. The adipose MSCs are easily obtained from adipose tissue with high yield and strong suppressive capabilities [64]. The umbilical cord MSCs are easy to get after birth, they have high self-renewable and differentiation capacities. The synovial-MSCs have high proliferative and differentiation capacities and very low immunogenicity [64].

MSCs from BM from mouse and from human were the first to be identified and are the most studied [54, 58]. Adipose stem cells (ASCs) were first identified as stem cells in 2001, capable to differentiate into cartilage, bone and adipose cells [65].

The age of the donor reflects on the differentiation potential of the cells [66]. Umbilical cord MSCs (UC-MSCs) showed to be highly proliferative and with differentiation potentials [67]. The drawbacks of the BM-MSCs are that the procedure to obtain the cells is painful, costly and does not yield high number of cells for cell therapy [49, 68, 69]. Besides, the procedure for BM isolation can result in potential infections [54].

Synovial-MSCs (S-MSCs) isolated and characterized first in 2001, are also promising tools for the treatment OA due to their natural homing in this site [70]. They were shown to have high chondrogenic differentiation capacities, high expression of type II collagen, compared to other sources of MSCs [71, 72]. S-MSCs are isolated in low numbers from different sites, like styloid fossa and paralabral synovium [73, 74]. They have low immunogenicity with high proliferation potentials [75, 76].

The adipose MSCs (A-MSCs) represent many advantages compared to other sources of the MSCs. They are abundant in the adipose tissue, adipose tissue is easier to get compared to BM, they have strong immunosuppressive capacities [47].

More research is needed to unveil the differences in the traits of A-MSCs isolated from different sites and their implications in therapy. Previously, it was shown that A-MSCs could differentiate from one site to another in term of their cell markers. The A-MSCs isolated from abdominal adipose tissue had high expression in CD31, CD45 and HLA-DR compared to the cells isolated from orbital adipose and had lower expression of CD73, CD90, CD105 and CD146 [77]. MSCs from adipose tissue represent a good and a promising alternative to bone marrow MSCs. They share the same phenotype as MSCs from bone marrow apart from few. They differentiate similarly to BM-MSCs into the three lineages, chondrocytes, adipocytes and osteocytes. Most importantly, they are easily obtained from liposuction aspirate of patients undertaking plastic surgery [65]. Adipose tissue contains greater number of stem cells than bone marrow (10 times higher) [59]. A large number of cells are required for transplantation. Stem cells isolated from adipose tissue or another source like bone marrow, need to be scaled up. At least 2x106 of MSCs is required per kilogram body weight [78].

MSCs from different sites (BM, adipose) tissue are heterogeneous cell population [79]. Although MSCs from bone marrow and from adipose tissue have similar differentiation potential, there are minor differences. ASCs showed higher chondrogenic potential than MSCs from bone marrow [80]. Other study however, showed no significant differences between these two populations for chondrogenic differentiation in 2D culture but the BM-MSCs showed higher chondrogenic differentiation in 3D culture [81]. Proliferative capacities and osteogenic differentiation of the MSCs from bone marrow are reduced with age [82]. According to previous report, MSCs can be affected differently depending on the disease. For example, it was reported that the BM- MSCs from osteoporotic patients had reduced osteogenic activity [82], whereas the BM-MSCs from OA patients did not show any differences with the normal patient [83]. In another, the chondrogenic and adipogenic differentiation of the MSCs were reduced in OA patients but not the osteogenic differentiation [84].

The expression of CD36 differed between donors from no expression to highly expressed [85]. However, all ASC extracted from different donors showed the expression of the CD90, CD73 and CD105. ASCs markers can change in expression depending on the age of cells in culture. CD106 is expressed in MSCs from bone marrow but not in ASCs where this latter expresses CD49b [52]. ASCs and BM-MSCs express CD29 (beta-1 integrin, important factor in angiogenesis) [74] and CD44 (hyaluronate receptor, important for neoextracellular matrix) and CD49e (alpha-5 integrin, important for cell adhesion to fibronectin) [59]. ASCs and BM-MSCs have been shown to secrete angiogenic growth factors, like VEGF, P1GF, bFGF, angiogenin, GM-CSF, MCP-1 and SDF-1alpha (Rehman, 2004; Kinnaird et al.; 2004), these could be involved in increased angiogenesis in ischaemic tissue [59]. CD117 (stem cell factor) a marker for totipotency and pluripotency, was expressed in ASCs and BM-MSCs [85]. Based on the International Society for Cellular Therapy, the minimum criteria to define mesenchymal stem cells are CD105 and CD90, their potential to differentiate to adipocytes, chondrocytes and osteocytes and they are plastic adherent [86]. The expression of the CD34 is only reported on the adipose derived mesenchymal stem cells but the expression decreases in culture [87, 88] and the expression of CD105 increases [89] (Braun et al.; 2013). CD34 is expressed on ASCs before they are isolated from the stromal vascular fraction (SVF) [89] (Braun et al., 2013). The International Society for Cellular Therapy (ISCT) and the International Federation for Adipose Therapeutics

and Science (IFATS) agreed on these changes in phenotype of ASCs in SVF (uncultured) or when cultured [90]. The SVF is referred to the cellular pellet containing ASCs and endothelial progenitor cells without adipocytes and immune cells, [89]. When the SVF is plated, the ASCs adhere to the surface and the rest of the cell population including non-adherent and non-proliferating cells, are removed [89]. ASCs could be isolated from SVF by culture or by magnetic-activated cell sorting (MACS) [89]. The advantage of the MACS isolation is cells can be prepared in few hours as required in clinical application. When cells need to be expanded, isolating the ASCs with culture is the best method. ASCs and SVF could be used for transplant but the number of cells obtained from patients could limit the use of SVF. ASCs could be expanded to a large number of cells, which could be used for autologous transplant or banked for allogeneic transplant [89]. These differences could be due to site differences or due to the lack of standardized isolation method [91].

14.1 Clinical trials with ASCs

MSCs showed to have a big potential in clinical applications. The first clinical trials run on the application of bone marrow MSCs in patients with Hurler syndrome and metachromatic leukodystrophy (MLD) after allogeneic hematopeitic stem cells transplant, showed no toxicity secondary to the bone marrow MSCs transplant and the recovery of some of the symptoms were suggested to be due to the transplanted MSCs [92]. Since then, hundreds of MSCs clinical trials were run on many conditions including neurological diseases, cardiovascular, autoimmune and bone and cartilage diseases [93]. So far, there have been few approved clinical application for the MSCs in different countries. Few examples, are the application of allogeneic MSCs for the treatment of graft versus host disease (GVHD) in Japan, autologous bone marrow MSCs for amyotrophic lateral sclerosis and autologous adipose MSCs for Chron's fistula, human umbilical cord blood-derived MSCs for osteoarthritis in South Korea, and in Europe the application of allogenic adipose MSCs for the treatment of fistulas in Crohn's disease [70].

There are few procedures for the treatment of cartilage injuries; the arthroplasty, microfat grafting and the autologous transplant of chondrocytes but a curative therapy still need to be developed [94, 95].

The autologous chondrocytes implantation is a surgical procedure that involves the isolation of autologous chondrocytes, expanding them in vitro then transplanting them back to the patient [96]. The application of MSCs for knee repair showed to be more effective with fewer side effects from the surgical procedure of the isolation of chondrocytes [96]. There are many clinical trials on the applications of the MSCs for OA [70].

OA is a very common condition in adults, which affects articular cartilage, subchondral bone, synovial tissue and meniscus of the joint. This leads to cartilage degeneration, osteophytes formation, subchondral sclerosis and synovial hyperplasia [97]. OA may be caused by joint injuries, obesity, aging and could be inherited condition [97].

Previous applications of MSCs for cartilage repair showed good outcomes. The BM-MSCs transplant in patients for defects in their knee cartilage and in athletes with the defect in femoral cartilage showed a good recovery of their functions [98].

MSC isolated from bone marrow, adipose tissue, umbilical cord, synovial membrane were previously used for the treatment of OA [64].

Since the discovery of ASCs, they have been used in many clinical trials for different diseases [90]. Success has been reported from different clinical trials using ASCs, but the mechanism of action is still not clear on whether cells would differentiate into the tissue or modulate the immune system [89]. Direct application of the

human ASCs improved cardiac function when injected in animals with myocardial infarction [99]. This effect was believed to be due to trophic factors released by the stem cells and differentiation of the ASCs. Local administration of ASCs accelerated the wound healing in normal and diabetic animals through differentiation into epithelial and endothelial lineage and neovascularization [100]. ASCs were applied to an injured skin due to radiotherapy, showed good healing process compared to the control [101]. ASCs although they have been used successfully in clinical trials, but their effects are not always achieved. There are many reasons that could impact the success of the use of ACS from one trial to another like cell separation, delivery methods, cell homing, engraftment and their survival [89]. Other factors might have an impact on the success of the use of ASCs like the type of liposuction procedure, site of liposuction, age of the patient and the body mass index (BMI). These factors are being investigated. Extraction of fat with different techniques might have an impact on cell viability of stem cells, their proliferation and the phenotype due to the trauma generated during the procedure. It is important to examine the different liposuction techniques and their impact on the ASCs phenotype, proliferation, and stress level for downstream applications either in research or in clinical applications.

15. Conclusion

Knee osteoarthritis is most prevalence musculoskeletal disease which cause functional limitations and affect a person's quality of life, there are known factors related to underling OA pathology such as Obesity, female gender and repetitive trauma to knee, Radiological finding such as subchondral cyst and narrowing of joint space, will support clinical diagnosis and staging of the disease using Kellgren and Lawrence staging system. Which will dictate the treatment modalities, in early stages conservative treatment such as modification of life style, rehabilitation, NSAID. Where advance stage surgical interventions ranging from arthroscopic debridement and lavage in case of blockage to total knee arthroplasty, which is being standard of care in severe condition.

Evolution of fat graft application in wide spectrum of clinical applications and have shown promising outcome to alleviate OA symptoms, where its contain adipocyte stem cells which known with reparative and regenerative process with presence of signal cofactors such as platelets derived growth factors and other growth factors to repaired damaged cartilage, fat graft being harvested from donor site mostly from abdomen, then;lipoaspirate will undergo further processing to isolate pure fat out from oil and fluid layer, then fat graft filtered with connector to reach pure micro fat graft ready to inject into affected knee under sterile process with local anesthesia.

Numerous clinical trials conducted to examine efficacy of microfat graft and ASC injection into knee with OA which demonstrated improvement in the overall condition, and further research with larger samples being in the process of publication to support clinical application and examine safety of patients.

DOI: http://dx.doi.org/10.5772/intechopen.99370

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