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Adipose Tissue-Derived Mesenchymal Stem Cells: A Novel Therapy in Orthopedics

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Abstract

Background: Mesenchymal stem cells can differentiate into a variety of cell types. They have the ability to release bioactive molecules, making them attractive tools in cell-based therapies. Adipose tissue is an ideal source of autologous mesenchymal stem cells for its abundance and easy access. Mesenchymal stem cells are routinely obtained enzymatically from lipoaspirate as stromal vascular fraction.

Objectives: The aim of this review is to investigate the advantages of adipose derived stem cells in orthopedic application.

Methods: Randomised controlled trials (RCTs) and non-RCTs, in addition to cohort and case control studies, as well as case series were included. Search language was restricted to English and time starting from year 2012. Only published papers were eligible.

Results: Thirteen articles were included in the review, they consisted of 1 RCT, 4 nRCTs, 1 prospective comparative study, 1 longitudinal cohort study, 2 case control studies, 3 case series and one case report. The number of patients injected with stem cells ranged between 12 and 1128. A total of 1482 patients were treated with adipose derived stem cells

Conclusion: The use of adipose derived stem cells had shown to be safe and effective in bone and cartilage repair.

Keywords: Mesenchymal stem cells; Adipose tissue; Regenerative medicine; Orthopedic application

Introduction

Regenerative medicine is an emerging field of multidisciplinary science. This evolving field is taking over the recent medical era as it combines growth factors, biomaterials, and stem cells to repair failing organs relying on fast and efficient tissue growth [1,2]. Regenerative medicine is being applied in orthopedics. It is being used to repair bone, tendon and cartilage damage [3].

Regenerative medicine is divided into two different techniques, the first is cell therapy and the second is tissue engineering. In cell therapy, a cell suspension is injected into the damaged tissue or blood circulation. This is used to repair damaged tissues to regain their function. However, this approach is insufficient to replace organs or regenerate large tissue defects. An alternative approach is tissue engineering. Yet, it is more complex and it is being barely used in clinical practice due to the issue of vascularization [3].

Mesenchymal Stem Cells

Regenerative medicine applications require a reliable and ideal source of stem cells, besides the needed development of cytokines factors and the biomaterial support [1]. On the other hand, both clinicians and researchers still face the challenge of finding available stem cells useful for regenerative medical practices. MSCs are multipotent cells, available in numerous tissues in one's body, such as bone marrow, muscle, adipose tissue, dental pulp, fetal organs, connective tissue, skin, placenta, blood, cord blood, synovium, periosteum, and perichondrium [2,4-23]. They are characterized by their ability to self-renew or differentiate *ex vivo* or *in vitro* into several cell lineages [1]. These cell types include adipocytes, chondrocytes, osteoblasts, and myoblasts [24-39]. In addition, MSCs have the ability to release bioactive molecules. Due to their proliferative and multi-potency differentiation, MSCs are considered to be strong pro-inflammatory process inhibitors that stimulate anti-inflammatory mechanisms, promising to serve in regenerative medicine and inflammatory disease treatment [25]. In this context, research is focusing on the study of the isolation, manipulation, and expansion of MSCs [25].

Adipose Tissue Derived Stem Cells

Researchers investigated and tested clinically the disadvantages of the bone marrow derived mesenchymal stem cells (hBMSCs) by enzymatic lipoaspiration process [40]. The aforementioned procedure is painful and invasive [40]. Its cell population yield is low and the cell fate *in vivo* is uncertain, especially that the delivery efficiency is reduced [20,40-46]. In addition, the cryogenic preservation followed by thawing of enzymatically aspirated fat tissue from bone marrow lead to a relatively negligible cell survival *ex vivo* [40,46]. Cell deterioration, decreased multipotency, as well as diminished proliferation potential are exhibited, increasing the chance for viral recipient tissue infection and morbidity [13,26,28,33,47-49].

In order to overcome the above hurdles, alternative sources of MSCs were sought. Adipose tissue of abdomen, thigh and hips serves as an ideal source of mesenchymal stem cells because of its availability and accessibility [26,27,29,30,40,49]. Adipose tissue derived stem cells are being employed in regenerative medicine due to their ability to differentiate into bone, tendon, cartilage, muscles and other tissues.

Non-enzymatic Isolation

Adipose tissue, or lipoaspirate, obtained from liposuction is

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digested with collagenase to release MSCs called adipose tissue-derived stem cell (ASCs). The ASCs undergo several rounds of centrifugation and dilution through which collagenase is washed off and the remaining product is the stromal vascular fraction (SVF) [35,50-54]. SVF has been used as a source of cells useful for tissue regeneration. The most common isolation technique to obtain SVF is the enzymatic digestion which consists of washing, treatment with enzymes, erythrocyte lysis, and cryopreservation or culture expansion [55-64]. This technique is costly and might jeopardize the safety as well as the efficacy of the lipoaspirates [4,65]. When compared to enzymatically derived MSCs, mechanically derived MSCs have superior differentiation potential with larger secretome content and more diverse exosome content without disruption of vascular stroma [66,67]. This method is purely mechanical with no chemical additives used; thus, showing promising results that power the method of non-enzymatic harvesting of stem cells derived adipose tissue [19,68-72].

Application of Adipose Derived Stem Cells in Orthopedics

The aim of this review is to investigate the advantages of adipose derived stem cells in orthopedic application. Randomised controlled trials (RCTs) and non-RCTs (nRCTs), in addition to cohort and case control studies, as well as case series were included. Search language was restricted to English and time starting from year 2012. Only published papers were eligible.

Study Characteristics

Thirteen articles were included in the review, they consisted of 1 RCT, 4 nRCTs, 1 prospective comparative study, 1 longitudinal cohort study, 2 case control studies, 3 case series and one case report [68,73-84]. The number of patients injected with stem cells ranged between 12 and 1128. A total of 1482 patients were treated with adipose derived stem cells. Table 1 summarizes the articles.

Rheumatoid Arthritis

Adipose derived stem cells have shown therapeutic effect *in vitro* [85]. They inhibit T-cell proliferation, produce inflammatory cytokines, and generate antigen-specific regulatory T-cells. Thus, they suppress responses of collagen II-reactive T-cells from patients with rheumatoid arthritis [85]. Alvaro-Gracia JM et al. conducted a randomized, placebo-controlled trial to assess the safety and tolerability of the intravenous administration of allogeneic expanded adipose-derived stem cells in patients with refractory rheumatoid arthritis. Three cohorts received treatment with doses of 1, 2 and 4 million cells/kg at days 1, 8 and 15 while the fourth cohort received placebo. They concluded that the intravenous administration of adipose derived stem cells was well tolerated and was not associated with dose-related toxicity. Signs for a potential therapeutic effect of these cells were observed [73].

Osteoarthritis

Intra-articular injection of adipose derived stem cells was shown to reduce synovitis, osteophyte formation, and cartilage degeneration in animal models [86,87]. Pers YM et al conducted a clinical trial to assess the safety and efficacy of intra-articular injection of autologous ASCs in patients with knee osteoarthritis. Eligible patients were allocated to three treatment groups (no placebo) with different doses (2, 10 and 50 million cells). They concluded that the technique was safe and well tolerated in patients with knee osteoarthritis [74].

Michalek J et al. carried out a case control study to evaluate the safety and clinical efficacy of autologous stromal vascular fraction (SVF) cells in patients with grade 2-4 degenerative osteoarthritis mainly of large joints (hip and knee) in addition to medium joints (elbow and wrist) as well as small joints (hand and foot). Single injection of adipose-derived SVF cells was given intra-articularly or peri-articularly to the synovial stromal tissue in close proximity of the joint. The technique was safe and clinically effective. This was demonstrated by the improved quality of life of patients [67].

Koh YG et al. carried out a prospective comparative study during which patients with a single International Cartilage Repair Society grade III/IV symptomatic cartilage defect on the femoral condyle were randomized into two groups. The first group received adipose derived stem cells with fibrin glue and micro fracture treatment whereas the second group received micro fracture treatment alone. The group that received stem cells and micro fracture with fibrin glue had improved radiologic, pain and symptom scores with no differences in activity, sports, or quality-of-life scores in comparison to the other group [78].

Jo CH et al. conducted a phase I/II clinical trial to assess the safety and the efficacy of intra-articular injection of autologous adipose tissue derived mesenchymal stem cells in patients with knee osteoarthritis. Phase I consisted of three cohorts; with three patients each. Patients in each dose group received 10, 50 and 100 million cells in 3 mL of saline. Three patients in each cohort were followed up for 28 days after injection. A safety review was done and phase II was started including 9 patients receiving 100 million cells. The authors concluded that intra-articular injection of 100 million adipose stem cells into the osteoarthritic knee improved function and reduced knee pain without causing adverse events. Radiological, histological and arthroscopic measures showed decreased articular cartilage defects by regeneration of hyaline-like articular cartilage [76].

Bui KH et al. performed a clinical trial to evaluate the efficiency and side-effects associated with the injection of SVF (obtained from adipose tissue of the belly) and platelet rich plasma in knee osteoarthritis. All patients had improved joint function and reduced pain scores. MRI findings showed significant improvements and increased thickness of cartilage layer. There were no complications with respect to infection or graft rejection [77].

Koh YG et al. described a series of elderly patients with knee osteoarthritis who received injections of adipose-derived stromal vascular cells after arthroscopic lavage. They concluded that the technique was effective in reducing pain, cartilage healing and improving function [81].

Pak J et al. carried out a retrospective cohort study in which patients were treated with autologous adipose derived stem cells with platelet-rich plasma for hip, knee, low back and ankle procedures. They concluded that the technique was safe when used as percutaneous local injections and there were no neoplastic complications [79].

Koh YG et al. conducted a case control study to assess the safety and effectiveness of mesenchymal stem cells (MSCs) derived from the infrapatellar fat pad when percutaneously injected into arthritic knees. Patients in the study group received a mean of 1.89 million stem cells

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Table 1: Application of adipose derived stem cells in orthopedics.

Author	Diagnosis	Treatment	n patients	Conclusion
Álvaro-Gracia JM et al. [74] (multicentre, dose escalation, randomised, single blind, placebo-controlled phase Ib/IIa clinical trial)	Refractory rheumatoid arthritis	3 intravenous infusions of allogeneic adipose-derived mesenchymal stem cells: 1 million/kg, 2 million/kg, and 4 million/kg or placebo, on days 1, 8 and 15, and they were followed for therapy assessment for 24 weeks	53	Technique was well tolerated, without evidence of dose-related toxicity. A trend for clinical efficacy was observed.
Pers YM et al. [75] (phase I, prospective, bicentric, single-arm, open-label, doseescalating clinical trial)	Symptomatic primary knee osteoarthritis	Single intra-articular injection of autologous adipose-derived stromal cells	18	Technique was safe and well tolerated. This study also provides encouraging preliminary evidence of efficacy
Michalek J et al. [68] (case control)	Grade 2-4 degenerative osteoarthritis of 1-4 large weight bearing joints (including hip and knee) and additionally 0-8 other joints (including shoulder, elbow, wrist, hand, ankle, foot)	Single injection of autologous adipose-derived stromal vascular fraction (SVF) cells was administered intraarticularly or periarticularly to the synovial stromal tissue in the close proximity of the joint	1128 (1856 joints)	Technique was safe and clinically effective leading to improved quality of life
Koh YG et al. [79] (prospective comparative study)	Single International Cartilage Repair Society grade III/IV symptomatic cartilage defect on the femoral condyle	Cell suspension had SVF containing ADSCs. Implantation of cell-thrombin-fibrinogen suspension into each well on the cartilage lesion surface	80	Compared with microfractrure (MFX) alone, MFX and ADSCs with fibrin glue provided an improved radiologic appearance of lesions and improved KOOS pain and symptom subscore improvements, with no differences in activity, sports, or quality-of-life subscores
Lee SY et al. [76] (Non-randomized clinical trial)	Lateral epicondylosis	Under ultrasound guidance, allogeneic adipose-derived mesenchymal stem cells mixed with fibrin glue were injected into the hypoechoic common extensor tendon lesions	12	Allogeneic adipose-derived mesenchymal stem cells therapy was safe and effective in improving elbow pain, performance, and structural defects for 52 weeks
Jo CH et al. [79] (phase I/II clinical trial with no active control)	Knee osteoarthritis	ADSCs were prepared from abdominal subcutaneous fats by liposuction and injected into knee joint through medial portal	18	Technique improved function and pain of the knee joint without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage
Bui KH et al. [78] (clinical trial)	Knee osteoarthritis at grade II and III	Adipose tissue was isolated from the belly and used for extraction of the SVF which was mixed with activated platelet-rich plasma before injection	21	Significant improvements and increased cartilage layer thickness were noted. There were no side-effects or complications
Pak et al. [85] (case report)	Avascular necrosis of femoral head	Adipose stem cells containing SVF was mixed with platelet rich plasma and hyaluronic acid. and injected into the diseased hip under ultrasound guidance	1	Pain scores, range of motion and MRI at 18 and 21 months after treatment indicated complete resolution of avascular necrosis of femoral head.
Pak J et al. [80] (retrospective cohort)	Chronic or degenerative joint disease	Mixture of adipose tissue-derived stem cells, platelet-rich plasma, hyaluronic acid, and $CaCl_2$ was injected into the joint under the ultrasound guidance	91	Technique could be considered to be safe with no neoplasctic complications
Pak J et al [80] (case series)	Chondromalacia patellae	Lipoaspirates were obtained from lower abdominal subcutaneous adipose tissue. Stem-cell-containing SVF was mixed with calcium chloride-activated platelet rich plasma and hyaluronic acid and was injected under ultrasonic guidance into the retro-patellar joints	3	Technique was safe and helped in restoring damaged tissues (softened cartilages)
Koh YG et al. [82] (case series)	Knee osteoarthritis	Adipose-derived SVF cells were injected in the selected knees of patients after arthroscopic lavage	30	Technique was effective in cartilage healing, reducing pain, and improving function
Koh YG et al. [81] (case control)	Knee osteoarthritis	The MSCs derived from infrapatellar fat pad were injected in selected knees of patients	25	Technique was safe, and provided assistance in reducing pain and improving function
Pak J [84] (case series)	Osteonecrosis of femoral head	Stem cells were obtained from lipoaspirates of lower abdominal subcutaneous adipose tissue. The stem-cell-containing SVF was mixed with calcium chloride-activated platelet rich plasma and hyaluronic acid and the mixture was injected into the affected hip.	2	Improvement in the patients' MRI scans was noticed. Patients' pain scores were reduced.

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with 3.0 mL of platelet-rich plasma and injected in the selected knees of patients while patients in the control group received platelet rich plasma that does not contain stem cells. There were no complications such as infection, fever, hematoma, tissue hypertrophy, adhesion formation observed among the study subjects. The authors concluded that the therapy reduced pain and improved patients' function [80].

Lateral Epicondylosis

Lee SY et al. evaluated the safety and efficacy of allogeneic adipose-derived mesenchymal stem cells for the treatment of lateral epicondylosis. Allogenic adipose derived stem cells were mixed with fibrin glue and injected into the hypoechoic common extensor tendon lesions under ultrasound guidance. Patients had reduced pain scores, improved elbow performance scores and decreased tendon defects. Within 52 weeks after the injection [77].

Chondromalacia Patellae

Pak J et al. evaluated the injection of adipose derived stem cells in series of three patients with Chondromalacia Patellae. The stromal vascular fraction was separated from the lipoaspirates by centrifugation after treatment with collagenase. The stem-cell-containing stromal vascular fraction was mixed with calcium chloride-activated platelet rich plasma and hyaluronic acid. The mixture was injected under ultrasonic guidance into the retro-patellar joints of the patients. The pain scores improved 50-70% one month after the injection and 80-90% after three months. The pain improvement persisted over one year. There were no serious side effects. The repeated magnetic resonance imaging scans at three months showed restoration of the damaged tissues (softened cartilages) on the patellae-femoral joints [82].

Osteonecrosis of Femoral Head

Pak J et al. evaluated the effect of adipose tissue-derived stem cells and platelet-rich plasma on to the regeneration of medullary bone-like tissue and long-term reduction of pain in two patients with femoral head osteonecrosis. Stem cells were obtained from lipoaspirates of lower abdominal subcutaneous adipose tissue. The stromal vascular fraction was separated from the lipoaspirates by centrifugation after treatment with collagenase. The stem-cell-containing stromal vascular fraction was mixed with calcium chloride-activated platelet rich plasma and hyaluronic acid. The mixture was injected into the affected hip. The diseased hip was re-injected with calcium chloride-activated platelet rich plasma on weekly basis for 4 weeks. The physical therapy testing, visual analog scale score, and Harris Hip score of the two patients improved after the treatment. Both patients showed improvement in their MRI scans, evidenced by positive T1 signal changes consistent with medullary bone regeneration. Moreover, the long-term reduction in hip pain was correlated with the MRI findings indicative of bone regeneration. The two cases demonstrated the presence of sustained, regenerated medullary bone-like tissue in severely necrotic femoral heads and suggest that this simple and minimally invasive procedure may be a promising therapy for patients with femoral head osteonecrosis [83].

Another report by Pak J et al. described a single case of early stage a vascular necrosis of the femoral head. Adipose derived stem cells containing stromal vascular fraction were mixed with platelet rich plasma and hyaluronic acid. The mixture was injected into the diseased hip under ultrasound guidance. The affected hip was reinjected weekly with additional platelet rich plasma for 4 weeks. The patient was followed-up MRI scans at 3, 18, and 21 months after treatment. Visual Analogue Scale, Walking Index, Functional Rating Index, Harris Hip Score, and Range of Motion assessments were also noted. The patient's severe hip pain was considerably improved at 3 months after treatment. Pain scores, range of motion and MRI at 18 and 21 months post treatment indicated complete resolution of a vascular necrosis of the femoral head [84-87].

Conclusion

The use of adipose derived stem cells had shown promising results in bone and cartilage repair. Further clinical trials are needed to confirm the short and long term efficacy of adipose derived stem cells in various orthopedic applications.

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