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Adipose-derived stem cells in the treatment of knee osteoarthritis: from extraction methods to the preparation of the transplant

Células-tronco derivadas do tecido adiposo no tratamento da osteoartrite do joelho: desde os métodos de extração até a preparação para o transplante

Células madre derivadas de tejido adiposo en el tratamiento de la osteoartritis de rodilla: desde los métodos de extracción hasta la preparación del trasplante

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Abstract
Introduction: Recent studies have investigated the use of adipose tissue as source of mesenchymal stem cells in the treatment of knee osteoarthritis in humans. However, there are still several protocols being performed. Objective: Analyze the protocols published in the literature in the last ten years and to investigate how they are being carried out and if they are following the criteria adopted by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Methodology: Articles from the PubMed, ScienceDirect and Lilacs database published in January / 2010 until the present time, which were evaluated in order to investigate the use of adipose-derived stem cells in the treatment of knee osteoarthritis. Results: Thirty four articles were evaluated in its entiraty. The abdominal area was the most choosen to do the liposuction, however the quantities of adipose tissue removed and the number of cells transplanted was variable. It is hightlited the enzimatic digestion of adipose tissue with collagenase as extraction method. Only 14 articles complied all the 3 criteria required to prove the real presence of mesenchymal stem cells in the samples that was transplanted. However, all the articles showed improvement of function and pain. Final considerations: Thus, even the results found are promising, the evidence is still limited in humans and the variability of the methodology makes it difficult to standardize the technique, also its implementation as a reference in the treatment of knee osteoarthritis.
Keywords: Osteoarthritis; Knee; Mesenchymal stem cells; Adipose tissue.

Resumo
Introdução: Estudos recentes investigaram a utilização do tecido adiposo como fonte de células tronco mesenquimais no tratamento da osteoartrite de joelho em humanos. No entanto, ainda existe muita variabilidade nos protocolos existentes. Objetivo: Analisar os protocolos publicados na literatura nos últimos dez anos e investigar como estão sendo realizados e se seguem os critérios adotados pela International Federation for Adipose Therapeutics and Science (IFATS) e pela International Society for Cellular Therapy (ISCT). Metodologia: Artigos das bases de dados PubMed, ScienceDirect e Lilacs publicados em janeiro / 2010 até o momento, foram avaliados, a fim de, investigar o uso de células tronco derivadas do tecido adiposo no tratamento da osteoartrite de joelho.

Resultados: Trinta e quatro artigos foram avaliados na íntegra. A região abdominal foi a mais escolhida para a realização da lipoaspiração, porém as quantidades de tecido adiposo retiradas e o número de células transplantadas foram variáveis. A digestão enzimática do tecido adiposo com a colagenase durante o método de extração foi a forma mais utilizada. Apenas 14 artigos atenderam a todos os 3 critérios exigidos para comprovar a real presença de células tronco mesenquimais nas amostras transplantadas. No entanto, todos os artigos mostraram melhora da função e da dor. Considerações finais**:** Assim, mesmo com os resultados promissores encontrados, as evidências ainda são limitadas em humanos. A variabilidade da metodologia dificulta a padronização da técnica impedindo assim que este tipo de terapia se torne referência no tratamento da osteoartrite do joelho.
Palavras-chave: Osteoartrite; Joelho; Células tronco mesenquimais; Tecido adiposo.

Resumen
Introducción: Estudios recientes han investigado el uso del tejido adiposo como fuente de células madre mesenquimales en el tratamiento de la osteoartritis de rodilla en humanos. Sin embargo, todavía existe mucha variabilidad en los protocolos existentes. Objetivo: Analizar los protocolos publicados en la literatura en los últimos diez años e investigar cómo se están llevando a cabo y seguir los criterios adoptados por la International Federation for Adipose Therapeutics and Science (IFATS) y por la International Society for Cellular Therapy (ISCT). .Metodología: Se evaluaron artículos de las bases de datos PubMed, ScienceDirect y Lilacs publicados en enero de 2010 hasta la fecha para investigar el uso de células madre derivadas del tejido adiposo en el tratamiento de la osteoartritis de rodilla. Resultados: Treinta y cuatro artículos fueron evaluados en su totalidad. La región abdominal fue la más elegida para la liposucción, pero la cantidad de tejido adiposo extraído y el número de células trasplantadas variaron. La digestión enzimática del tejido adiposo podría estimularse con colagenasa como método de extracción. Solo 14 artículos cumplieron los 3 criterios requeridos para probar la presencia real de células madre mesenquimales en las muestras trasplantadas. Sin embargo, todos los artículos mostraron una mejora en la función y el dolor. Consideraciones finales: Así, incluso los resultados encontrados son prometedores, la evidencia aún es limitada en humanos y la variabilidad de la metodología dificulta la estandarización de la técnica y su aplicación como referencia en el tratamiento de la artrosis de rodilla.
Palabras clave: Artrosis; Rodilla; Células madre mesenquimales; Tejido adiposo.

1. Introduction

Osteoarthritis (OA) is a complex disease with unknown etiology that affects several joints, it is one of the main causes of chronic pain and decreased independence of the elderly individuals (Cooper et al., 2014). Nowadays the available techniques for OA treatment provide relief of symptoms, but not the regeneration of the injured tissue. Cartilage regeneration is defined as the restoration of articular cartilage in its original condition at histological, biochemical and biomechanical levels, becoming indistinguishable from healthy cartilage (Mollon et al., 2013). In front of this, the currently researches of cell therapies direct their focus in the construction of a biological substitute that incorporates the articular cartilage, being capable of synthesizing the cartilaginous matrix (Stromps et al., 2014). An innovative treatment that has shown good results in the OA treatment and has been carried out in different parts of the world, is the cell therapy with adipose-derived stem cells (ADSCs). This treatment showed a protective effect against joint degeneration, improvement of pain, cartilage volume and functionality of treated individuals (Pak et al., 2017).

The adipose tissue has the simplest technique of cell extraction among the mesenchymal stem cells (MSCs) sources. It is made with a minimally invasive procedure that allow the separation of a larger amount of cells if compared with the other tissue sources (Labusca et al., 2013). Many studies are still performed based on data presented in the literature, making their protocols often not yet standardized for clinical application. Details, such as how the material is lipoaspirate, washed and digested, makes several cell types available for handling, necessitating criteria for classification and separation of the ADSCs. Due to these factors, the use of ADSCs is currently not considered the standard treatment for OA. According to the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) the main prerequisites to be considered in ADSCs are: adhesion in monolayer culture, differentiation in chondrocytes, osteocytes and adipocytes *in vitro* and the presence of markers CD73, CD90 and CD105, and absence or negativity of the CD45, CD34, CD14 (or CD11b), CD79α (or CD19), and HLA-DR (Bourin et al., 2013; Dominici et al., 2006). Therefore, the

intention of this review is to analyze published articles that claim to use ADSCs as a treatment for knee with osteoarthritis and to verify if all the listed prerequisites have been fulfilled, demonstrated and explained in full in the works, in order to encourage this therapy to be become the gold standard for the treatment of this pathology.

2. Methodology

Articles from the PubMed, ScienceDirect and Lilacs database of the last ten years (January / 2010 until the present time) were evaluated to investigate the use of adipose-derived stem cells in the treatment of OA knee. First, the search was performed using the boolean operator AND between the descriptor *knee osteoarthritis* and the following descriptors: *adipose-derived stem cells* and *mesenchymal stem cells* or *mesenchymal stromal cells*.

The articles were selected by title and abstract using the following inclusion criteria: a) the experiment should have been performed in humans; b) individuals treated should have OA knee; c) studies should involving the use of adipose-derived stem cells; d) the articles should have been published in January / 2010 until the present time; e) the article should be in English. The following exclusion criteria were adopted: a) animal experiments; b) cell therapies performed with mesenchymal stem cells from another tissue as source; c) review studies, editorials, comments, or letters d) aggressive treatment on the surface of the joint such as marrow stimulation and microfracture.

According to the eligibility criteria, the LCSA and SMC authors independently selected the studies in two stages, evaluating the title and abstract, and later, by reading the full text. The tables were completed with the interpretation of the information contained in the text of the articles, possible cited references, and also supplementary material when available. Disagreements were resolved by consensus.

3. Results

Screening for the choice of articles

A total of 7.354 articles was found (Pubmed : 922 ; Science direct: 5.074 and Lilacs: 858), of which 5.598 articles was published between 2009 and 2021 (Pubmed : 839; Science direct: 3.965 and Lilacs:794), 567 articles in duplicated were excluded. After a detailed reading of the abstracts and the application of the inclusion and exclusion criteria, 50 articles remained, which was read in their entirety. Then, another 16 studies was excluded because is not consistent with the established criteria, remaining in the evaluation a total of 34 articles. The selected articles were organized according the following items: Authors and year of publication; Nº of participants with OA knee; Control group; Removal sites of adipose tissue; Volume removed; Extraction method; Number of cells transplanted and Treatments (Table 1), Adhesion in monolayer culture, Immunophenotyping and Cell differentiation (Table 2).

Details and steps used in the extraction protocols of adipose-derived stem cells

Regarding the presence of a control group, just were found only in 6 studies that follow groups with treatments without ADSCs or SVF to compare the results (Table 1).

The target body areas for liposuction were the abdominal region in 25 articles, infrapatellar region in 2 articles, flank region in 1 article, thighs region in 3 articles and buttock region 7 articles. The amount of adipose tissue extracted during the liposuction procedure ranged from 5 until 360ml (Table 1).

Two categories of technique for processing adipose tissue were found in this review (Table 1):

- **Mechanical separation**: 1 by Cell-Innovations (Australia) standard ultrasonic cavitation proprietary protocol (Stromed), that used this process to obtain the SVF; 3 articles with the use of Processing cylinder Lipogems®, which is a closed device that

allows the separation and elimination of blood and impurities from the liposuction samples by extensively washing and agitation, thus the steel marbles present inside the device fragment the lipoaspirate and produce what will be used in the transplant; and 1 by centrifugation, the lipoaspirates were centrifuged at 3000 rpm / 3min to collect the fat phase.

- **Enzymatic digestion**: 3 articles used the Celution® Centrifuge, which is a closed system started with just one button and uses Celase® and Intravase® as enzyme and delivers the SVF at the end of the process to be used in the transplant. However, the majority (26 articles) performed the extraction with collagenase, but the concentrations used, collagenase unit, collagenase action time, centrifugations performed and types of manipulations, in the articles that presented or cited the method used, do not have standardization.

After the processing, 5 studies used as treatment micro-fragmented adipose tissue, 18 studies used stromal vascular fraction (SVF) and 12 studies used ADSCs culture expanded in the cell therapy, the period of culture ranged from 2 to 4 weeks before being injected into the joint (Table 1).

Table 1- Table of articles selected for full analysis of extraction methods.

Author and year of publication	Nº	Control group	Removal sites of adipose tissue	Volume removed	Extraction method	Number of cells transplanted	Treatments
(Koh & Choi, 2012)	50	yes	Infrapatellar	9.4 g	Enzymatic – 0.1% collagenase type 1	1.89×10^6 ADSCs*	**Knee arthroscopy surgery #** **Group control:** received 3 intra articular injections of PRP **Group treated:** received 1 intra articular injection of SVF + PRP, and more 2 intra articular injections of PRP
(Pak et al., 2013)	74 knees	no	Abdomen	100 ml	Enzymatic – 0.07% collagenase type 1	n/i	Received 1 intra articular injection of SVF + PRP + HA and more 4 intra articular PRP injections in the follow 4 weeks
(Koh et al., 2013)	18	no	Infrapatellar	9.1g	Enzymatic – 0.1% collagenase type 1	$1.18\ x10^6$ ADSCs	**Knee arthroscopy surgery #** Received 1 intra articular injection of SVF + PRP, and more 2 intra articular injections of PRP
(Jo et al., 2014)	18	no	Abdomen	n/i	Enzymatic – collagenase type 1(1mg/mL)	$1.0x10^7$ ADSCs* $5.0x10^7$ ADSCs* $1.0x10^8$ ADSCs*	**Knee arthroscopy surgery** **PHASE 1** **Group 1:** Received 1 intra articular injection of ADSCs ($1.0x10^7$ ADSCs**)** **Group 2:** Received 1 intra articular injection of ADSCs ($5.0x10^7$ ADSCs**)** **Group 3:** Received 1 intra articular injection of ADSCs ($1.0x10^8$ ADSCs) **PHASE 2** Another 9 patients received 1 intra articular injection with the high dose of ADSCs ($1.0x10^8$ ADSCs**)**

(Bui et al., 2014)	21	no	Abdomen	50-100 ml	Enzymatic – ADSC Extraction Kit (GeneWorld, Ho Chi Minh City, Vietnam)	n/i	Received 1 intra articular injection of SVF + PRP
(Koh et al., 2014)	56	no	Buttock	22.6 g	Enzymatic – 0.075% collagenase	3.8 x 10^6 ADSCs*	**Knee arthroscopy surgery #** Received 1 injection of ADSCs implanted at the site of the lesion
(Koh et al., 2015)	30	no	Buttock	140 ml	Enzymatic – 0.075% collagenase	4.04 x 10^6 ADSCs (9.7 % of 4.16 x 10^7 SVF) *	**Knee arthroscopy surgery** Received 1 injection of SVF + PRP applied at the site of the lesion
(Gibbs et al., 2015)	4	no	Abdomen	100 ml	Mechanic – Ultrasonic cavitation proprietary protocol "StroMed"	7.5x10^7 SVF 5x10^7 SVF 10x10^7 SVF 6x10^7 SVF	Received 1 intra articular injection of microfragmented adipose tissue + PRP and more 3 intra articular injections of PRP in the follow 3 months
(Kim, Choi, Suh, et al., 2015)	54	no	Buttock	140 ml	Enzymatic – 0.075% collagenase	4.2 x 10^7 SVF which contained an average of 3.9x 10^6 ADSCs *	**Knee arthroscopy surgery # Group 1:** Received 1 injection of SVF applied at the site of lesion **Group 2:** Received 1 injection of SVF + fibrin glue implanted at the site of lesion
(Kim, Choi, & Koh, 2015)	49	no	Buttock	140 ml	Enzymatic – 0.075% collagenase	4.6 x 10^7 SVF which contained an average of 4.3 x 10^6 ADSCs *	**Knee arthroscopy surgery #** Received 1 SVF injection + fibrin glue implanted at the site of lesion
(Kim, Kwon, et al., 2015)	40	no	Buttock	n/i	Enzymatic – 0.075% collagenase	4.07 x 10^6 ADSCs * 3.96 x 10^6 ADSCs *	**Knee arthroscopy surgery # Group 1:** received 1 injection of ADSCs + PRP at the site of lesion **Group 2:** received 1 injection of ADSCs + fibrin glue implanted at the site of lesion
(Fodor & Paulseth, 2015)	6	no	Abdomen, flanks, and or lateral thighs	150- 250 ml	Enzymatic – Collagenase type 1 200 CDU/mL	41x10^6 SVF 14x10^6 SVF / 2 knees 17.1x10^6 SVF 17.6x10^6 SVF 15.2x10^6 SVF / 2 knees 7.9x10^6 SVF	Received 1 intra articular injection of SVF
(Pak et al., 2016)	3	no	Abdomen	50 ml	Enzymatic – 0.07% collagenase type 1	n/i	Received 1 intra articular injection of SVF + PRP + HA, and 1 intra articular injection of PRP + HA per week for 3 weeks
(Pers et al., 2016)	18	no	Abdomen	60 ml	Enzymatic - 10 g of adipose tissue/ 34ml of collagenase	2 x10^6 ADSCs* 10 x 10^6 ADSCs* 50 x 10^6 ADSCs*	**Group 1:** Received 1 intra articular injection of ADSCs (2 x10^6 ADSCs**)** **Group 2:** Received 1 intra articular injection of ADSCs (10 x 10^6 ADSCs**)** **Group 3:** Received 1 intra articular injection of ADSCs (50 x 10^6 ADSCs)

(Kim et al., 2016)	20	no	Buttock	140 ml	Enzymatic – 0.075% collagenase	4.9 x10^7 SVF (4.4x 10^6 ADSCs)	**Knee arthroscopy surgery #** Received 1 injection of SVF + Fibrin glue implanted at the site of lesion
(Yokota et al., 2017)	13	no	Abdomen or thigh	200 ml or more	Enzymatic - Celase®, Intravase® Celution centrifuge IV	3x10^7 SVF	Received 1 intra articular injection of SVF
(Bansal et al., 2017)	10	no	Abdomen	100 ml	Enzymatic – Collagenase	1x10^6 /ml SVF	Received 1 intra articular injection of SVF + PRP
(Coughlin et al., 2017)	1	no	Abdomen	80-120 ml	Mechanic – Processing cylinder (Lipogems®)	n/i	**Knee arthroscopy surgery** Received 1 intra articular injection of microfragmented adipose tissue
(Jo et al., 2017)	18	no	Abdomen	n/i	Enzymatic – collagenase type 1 (1 mg/mL)	1.0x10^7 ADSCs* 5.0x10^7 ADSCs* 1.0x10^8 ADSCs*	**Knee arthroscopy surgery PHASE 1** **Group 1:** Received 1 intra articular injection of ADSCs (1.0x10^7 ADSCs**)** **Group 2:** Received 1 intra articular injection of ADSCs (5.0x10^7 ADSCs**)** **Group 3:** Received 1 intra articular injection of ADSCs (1.0x10^8 ADSCs) **PHASE 2** Another 9 patients received 1 intra articular injection with the high dose of ADSCs (1.0x10^8 ADSCs **)**
(Spasovski et al., 2018)	9	no	Abdomen	5 ml	Enzymatic - 0.1 % collagenase	0.5-1x10^7 ADSCs*	Received 1 intra articular injection of ADSCs
(Song et al., 2018)	18	no	Abdomen	50 ml	Enzymatic – 1 mg/mL de collagenase type 1	1 × 10^7 ADSCs 2 × 10^7 ADSCs 5 × 10^7 ADSCs	**PHASE 1** **Group 1 :** received 2 intra articular injection of ADSCs (1 × 10^7 ADSCs) one at the 3rd and the other at the 6th week after liposuction **Group 2 :** received 2 intra articular injection of ADSCs (2 × 10^7 ADSCs) one at the 3rd and the other at the 6th week after liposuction **Group 3 :** received 2 intra articular injection of ADSCs (5 × 10^7 ADSCs) one at the 3rd and the other at the 6th week after liposuction **PHASE 2** All pacients received the 3rd intra articular injection of ADSCs with the high dose (5 × 10^7 ADSCs) at the 48th week
(Pak et al., 2018)	3	no	Abdomen	100-110 g	Enzymatic – 10 mg of collagenase (5 mg of collagenase specific for	n/i	Received 1 intra articular injection of SVF + PRP + HA, and more 3 intra articular injection of PRP in the follow 3 weeks

					connective tissue/ 5 mg of collagenase specific for adipose tissue)		
(Panni et al., 2018)	52	no	Abdomen	40 – 60 ml	Mechanic – Lipogems® ortho kit	n/i	**Knee arthroscopy surgery #** Received 1 intra articular injection of microfragmented adipose tissue
(Cattaneo et al., 2018)	35	no	Abdomen	n/i	Mechanic - Lipogems® processing kit	n/i	**Knee arthroscopy surgery #** Received intra articular injection of microfragmented adipose tissue
(Hong et al., 2018)	16	yes	Abdomen	100-150 ml	Enzymatic – collagenase type 1	7.45 x 10^6 SVF	**Knee arthroscopy surgery** **Group 1:** received 1 intra articular injection of SVF in the left knee and 1 intra articular injection of HA in the right knee **Group 2:** received 1 intra articular injection of SVF in the right knee and 1 intra articular injection of HA injection in the left knee
(Freitag et al., 2019)	30	yes	Abdomen	60 ml	Enzymatic – 0.075% collagenase	103.9 x 10^6 ADSCs* 95.1 x 10^6 ADSCs* 102.6 x 10^6 ADSCs*	**Group 1:** Ongoing conventional conservative management only **Group 2:** 1 intra articular injection of ADSCs (103.9 x 10^6 ADSCs) **Group 3:** 2 intra articular injection of ADSCs (95.1 x 10^6 and 102.1 x 10^6 ADSCs)
(Roato et al., 2019)	20	no	Abdomen	35 ml	Mechanic-centrifugation of lipoaspirate	31 x10^6 SVF with 14.2% ADSCs	**Knee arthroscopy surgery** Received 1 intra articular injection of micro fragmented adipose tissue
(Lee et al., 2019)	24	yes	Abdomen	20ml	Enzymatic – collagenase type 1 (1 mg/mL)	1 × 10^8 ADSCs*	**Group control :** received 1 intra articular injection of saline solution **Group treated:** received 1 intra articular injection of ADSCs
(Lu et al., 2019)	52	yes	Abdomen	50 ml	Enzymatic – 1 mg/mL de collagenase type 1	5 × 10^7 ADSCs*	**Group 1:** received 4 intra articular injection of HA **Group treated:** received 2 intra articular injection of ADSCs and 2 Sham injections
(Onoi et al., 2019)	2	no	Thights	130ml	Enzymatic- Celution® Centrifuge (Cytori Therapeutics K.K., Tokyo, Japan)	1.2x10^6 ADSCs 5.5x10^6 ADSCs	**Knee arthroscopy surgery** Received 1 intra articular injection of SVF
(Yokota et al., 2019)	80	no	Abdomen	100 -200 ml	Enzymatic - collagenase (Amano Enzyme) and therymolysin (Amano Enzyme)	12.75 x 10^6 ADSCs* SVF: unknow	**Group 1:** Received 1 intra articular injection of ADSCs **Group 2:** Received 1 intra articular injection of SVF

(Lapuente et al., 2020)	50	no	Abdomen	60 ml	ADSC System commercial kit (Lyposmol Biotech, Madrid, Spain) collagenase type 1 and 2	3.21×10^6 SVF	Received 1 intra articular injection of SVF
(Tsubosaka et al., 2020)	57	no	Abdomen or breech	100–360 ml	Enzymatic-Celution® 800/CRS system (Cytori Therapeutics Inc., San Diego, CA) Celase® GMP	2.5×10^7 SVF	Received 1 intra articular injection of SVF
(Nasb et al., 2020)	96	yes	Abdomen	60 ml	Enzymatic – 0.075% collagenase	10 x10^6 ADSCs*	**Group 1:** Single intra-articular injection ADSCs with low-intensity pulsed ultrasound treatment for 20 min daily, 5 days a week, for up to 8 weeks. **Group 2:** Single intra-articular injection of ADSCs with shame ultrasound irradiation for 20 min daily, 5 days a week, for up to 8 weeks. **Group 3:** Normal saline with low-intensity pulsed ultrasound treatment for 20 min daily, 5 days a week, for up to 8 weeks.

n/i: not informed; ADSCs: adipose-derived stem cells; SVF: stromal vascular fraction; PRP: platelet-rich plasma ; HA: hialuronic acid ; * Before injection, bacteriologic tests were performed on the samples and # during the surgery was performed one or more of the following treatments: synovectomy; debridement; or excision of degenerative tears of the menisci, fragments of articular cartilage, chondral flaps, or osteophytes that prevented full extension. Source: Authors.

Details on the preparation process for adipose-derived stem cell transplantation

Regarding the cell amount used, 27 articles performed the cell count before the transplant. In articles that used SVF the amount of cel/ml ranged from 1x10^6 to 10x10^7 per application, and in those that used ADSCs the amount of cel/ml ranged from 2x10^6 to 1x10^8, this count was done in the moment of the application. The number of ADSCs per amount of adipose tissue may vary according of the region of the adipose tissue. In this review, the highest amounts of ADSCs in adipose tissue extracted from isolated areas were 1.89×10^6 in 9.4 g of Hoffa fat, 4.04×10^6 in 120 ml of the buttock and 6×10^4 abdominal region in 5 ml, this count was done before transplantation or expansion in culture (Table 1).

The number of applications of cellular therapy varied in the articles, 30 studies performed 1 application per participant of cells derived from adipose tissue, 3 studies performed 2 ADSCs injections, and 1 performed 3 ADSCs injections (Table 1).

Among the substances used concomitantly, together with the biological material of the proposed cell therapy, was found 7 articles using only platelet rich plasma (PRP), 2 with hyaluronic acid (HA), 3 articles with PRP+HA and 4 with fibrin glue. Arthroscopy surgery was also performed in 16 studies, in 7 it was used only for diagnostic and in the others during the surgery one or more of the following treatments was performed: synovectomy; debridement; or excision of degenerative tears of the menisci, fragments of articular cartilage, chondral flaps, or osteophytes that prevented full extension (Table 1).

On the criteria required by the IFATS and ISCT, 17 articles confirmed ADSCs adhesion in monolayer culture, 14 carried out the cell differentiation and 18 did the immunophenotyping of the where the positivity was found for the

mesenchymal cell markers CD73, CD90, CD105, CD49d, CD44, CD29 and CD271 the choice of markers for negativity varied CD14, CD34, CD31, CD45 and HLA-DR. However, only 14 studies complied the 3 criteria of IFATS and ISCT (Table 2).

Also was found in this review a variety of acronyms to designate MSCs derived from adipose tissue. The following names were raised: adipose tissue-derived stem cells (ADSCs); adipose derived mesenchymal stem cells (AD-MSCs); adipose-derived mesenchymal stem cells (haMSCs); adipose tissue derived mesenchymal stem cells (AD MSCs); adipose-tissue derived MSCs (ASCs). This variety interferes with demand in the literature, according to the International Fat Applied Technology Society the name to be adopted is "Adipose-derived stem cells" (ASCs) to identify these cells.

Table 2 – Prerequisites of International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT).

Author and year of publication	Adhesion in monolayer culture	Immuno phenotyping	Cell differentiation
(Koh & Choi, 2012)	n/i	n/i	n/i
(Pak et al., 2013)	n/i	n/i	n/i
(Koh et al., 2013)	n/i	n/i	n/i
(Jo et al., 2014)	yes	+ CD73 and CD90 - CD31, CD34 and CD45	Adipogenic Chondrogenic and Osteogenic
(Bui et al., 2014)	n/i	n/i	n/i
(Koh et al., 2014)	yes	+ CD90 and CD105 -CD34 and CD14	Adipogenic Chondrogenic and Osteogenic
(Koh et al., 2015)	yes	+ CD90 and CD105 -CD34 and CD14	Adipogenic Chondrogenic and Osteogenic
(Gibbs et al., 2015)	n/i	n/i	n/i
(Kim, Choi, Suh, et al., 2015)	yes	+ CD90 and CD105 -CD34 and CD14	Adipogenic Chondrogenic and Osteogenic
(Kim, Choi, & Koh, 2015)	yes	+ CD90 and CD105 -CD34 and CD14	Adipogenic Chondrogenic and Osteogenic
(Kim, Kwon, et al., 2015)	yes	+ CD90 and CD105 - CD34 and CD14	Adipogenic Chondrogenic and Osteogenic
(Fodor & Paulseth, 2015)	n/i	n/i	n/i
(Pak et al., 2016)	n/i	n/i	n/i
(Pers et al., 2016)	yes	+ CD105, CD90 and CD 73 - CD45, CD14 and CD 34	n/i
(Kim et al., 2016)	yes	+ CD105 and CD 90 - CD14 and CD 34	Adipogenic Chondrogenic and Osteogenic
(Yokota et al., 2017)	n/i	n/i	n/i
(Bansal et al., 2017)	yes	+ CD105, CD90 and CD73, - CD34, CD45 and HLA- DR	n/i
(Coughlin et al., 2017)	n/i	n/i	n/i

(Jo et al., 2017)	yes	+ CD73 and CD90 - CD31, CD34 and CD45	Adipogenic Chondrogenic and Osteogenic
(Spasovski et al., 2018)	yes	+ CD73, CD105, and CD90 - CD34 and CD45	Chondrogenic and Osteogenic
(Song et al., 2018)	yes	+ CD90, CD73, CD29 and CD49d - CD14, CD34, CD45 and HLA-DR	Adipogenic Chondrogenic and Osteogenic
(Pak et al., 2018)	n/i	n/i	n/i
(Panni et al., 2018)	n/i	n/i	n/i
(Cattaneo et al., 2018)	n/i	n/i	n/i
(Hong et al., 2018)	n/i	n/i	n/i
(Freitag et al., 2019)	yes	+ CD90, CD73 and CD 105 - CD14, CD19, CD34 and CD45	Adipogenic Chondrogenic and Osteogenic
(Roato et al., 2019)	yes	+ CD105, CD44, CD90, CD73 and CD271 - CD45	Chondrogenic and Osteogenic
(Lee et al., 2019)	n/i	+ CD90, CD73 - CD31, CD34 and CD45	n/i
(Lu et al., 2019)	yes	+ CD90, CD73, CD29 and CD49d - CD14, CD34, CD45 and HLA-DR	Adipogenic Chondrogenic and Osteogenic
(Onoi et al., 2019)	n/i	n/i	n/i
(Yokota et al., 2019)	yes	n/i	n/i
(Lapuente et al., 2020)	n/i	CD 31, CD45, CD34 E CD 146	n/i
(Tsubosaka et al., 2020)	n/i	n/i	n/i
(Nasb et al., 2020)	yes	CD44, CD 90, CD105, e CD 73 CD 34 and CD45	Adipogenic Chondrogenic and Osteogenic

n/i: not informed. Source: Authors.

4. Discussion

The ADSCs are considered multipotent with the ability to differentiate into a variety of cells, including osteoblasts, tendon, muscle, adipose tissue and chondrocyte (Toghraie et al., 2011). The number of these cells varies according to the location of the adipose tissue in the body, as well as its quality (Pak et al., 2017). It is known that ADSCs harvested from superficial abdominal regions have significantly less apoptosis than ADSCs harvested from another regions as upper arm, medial thigh, trochanteric, or superficial deep abdominal depots (Fraser et al., 2007). Also, with the fact that normally the fat of the donor are more disponible in this area, this justify why it was the most chosen area to made the liposuction among the articles in this review. However another factors can influence in the ADSCs quality like age of the donor, life style, chronic use of drugs (Kornicka et al., 2015), and the density and amount of extracellular matrix in the sample interferes directly in the digestion of this tissue (Pak et al., 2017), also during liposuction surgery, the non-use of vasoconstrictors in the emulsifying solution applied before the removal of the adipose tissue, favors a higher yield of ADSCs uptake.

The minority of studies obtained in this review , used the mechanic protocols for the ADSCs extracting, that obtain only the micro-fragmented adipose tissue, which is enriched in pericytes but has a smaller amount of stromal cells than the enzymatically digested tissues (Hudetz et al., 2017). The majority opted for the enzymatic digestion that was the first protocol published to the extraction of ADSCs, propose by Zuk, et al. (2001) with some modifications (Zuk et al., 2001). Nowadays the number of studies comparing the efficacy of this variety of methods is low, making ADSCs extraction standardization difficult. Even so, the use of collagenase is still considered the gold standard for obtaining these cell populations with more purity, and for presenting better yield in number and viability of ADSCs at the first moment after processing and also greater proliferation when compared to mechanic methods (Chaput et al., 2016; Markarian et al., 2014). However, in this review was observed the lack of a standardization of the concentrations, units / mg and the time of exposure, it may be factors that interferes directly in the effectiveness of the chosen protocol (Pagano et al., 2004), and these steps needs to receive attention to a greater investigation, because of the price of collagenase, fact that turns the enzymatic extraction expensive, because of this, always a new protocol appears or a new strategy to substitute the collagenase.

The increase in clinical applications for ADSCs in the OA knee increasingly demand the need for reliable information on the efficiency, cost and safety of automated equipment and manual techniques that were created to facilitate the separation of the stromal vascular fraction (SVF) from adipose tissue. The SVF is acquired at the first moment after the digestion of the liposuction material, in its composition exist hematopoietic stem cells, endothelial cells, pericytes, fibroblasts, monocytes, macrophages, and cells considered ADSCs (Bora & Majumdar, 2017). About 9.7% of SVF cells are considered stromal cell and progenitor in this initial pellet (Koh et al., 2015). The choice of SVF as cell therapy, is due to the rapid handling, extraction and implantation at the same time of surgery, even if the number of cells acquired is still relatively low for treatment of large lesions. The use of SVF or adipose tissue micro-fragmented is adopted because could get around the oversight by United States Food and Drug Administration (FDA) believing that it is considered “minimally manipulated” (Aronowitz et al., 2015). But, exist a document that clarifies this classification, in it FDA considers any minimal manipulation with adipose tissue derived and classify all methods of SVF isolation (enzymatic and mechanical), as “more than minimally manipulated” cells, so use SFV in any circumstances is considered as a drug (Food & Administration, 2014). Another point in this review that is concern, is that only 15 studies performed some test to detect bacteria, mycoplasma, or endotoxin in the samples before the transplant (to ensure the absence of contamination), even though these manipulations are said to be done in sterile environments and with good manners of practice, proving this safety in a clinical trial is very important.

There is a shortage in the literature of studies comparing effectiveness between the enzymatic method and new mechanical extraction protocols, and the difference between them regarding the proliferation yield of ADSCs. Another important point, in this present review, we observe that just one article (Roato et al., 2019) that used the mechanical extraction fulfilled the proposed criteria’s by ISCT and IFATS in the studies, the other 4 did not show that was check these criterias even has already been proven in the articles of the method´s origin (Stromed- (unpublished data); Lipogems® - (Bianchi et al., 2013). The fact that this criteria was not repeated and showed in the article of OA knee treatment, makes it difficult to know what was actually extracted in these treatments by the methods used and what is acting in the treatment of these patients, since the acquired SFV was applied to the OA knee shortly after the end of the processing and the cell count used was performed in a global way. The same happens in the articles that used the enzymatic protocol with collagenase, some mention Zuk, et al. (2001) (Freitag et al., 2019; Kim et al., 2016; Kim, Choi, & Koh, 2015; Kim, Choi, Suh, et al., 2015; Kim, Kwon, et al., 2015; Koh et al., 2015; Koh et al., 2014; Lapuente et al., 2020; Yokota et al., 2019) as reference but this did not justify the lack of these trials. This question was arisen because all authors mentioned the effect and presence of ADSCs in their treatments during the discussion, when in fact they did not prove completely the real presence of these cells in their samples. Regarding

the comparison of SVF and cultured ADSCs extracted by collagenase, only 1 article compared the effectiveness of these two biological materials, and significantly better results were observed in the use of cultured ADSCs (Yokota et al., 2019).

Regarding the number of ADSCs to be transplanted, a consensus has not yet been reached, but some studies have been investigating the difference in results between different amounts of ADSCs to enable the discovery of this (Jo et al., 2017; Jo et al., 2014; Pers et al., 2016; Song et al., 2018), on them the largest amounts of cultured cells transplanted, obtain better results, being the largest $1x10^8$ of ADSCs that maintained their treatment result for 2 years (Jo et al., 2017). In relation to the number of ADSCs applications, Song, et al, (2018) analyzed the effect of three injection of ADSCs and followed the results for 96 weeks, proved that these number of applications are safety and improved the pain, function and cartilage volume of the knee joint (Song et al., 2018). In another study to tested a higher number of applications the patients received five ADSCs injections, been one per month, was intended but ceased due to observed and reproducible moderate adverse events like increasing self-limiting pain because of sequential injections (Freitag et al., 2019).

It is known that the paracrine effects of MSCs generally occur by the presence of trophic factors include C granulocytes; interleukins-6, -7, -8 and -11; Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Transforming growth factor beta (TGFβ), Fibroblast growth factor (FGF-2) and many others (Brown, 2018) , MSCs "sense" and "signal" changes in the microenvironment where they reside and act through trophic, mitogenic, anti-scarring, anti-apoptotic, immunomodulatory, and anti-microbial actions (Caplan & Dennis, 2006). In the case of OA treatment the native cells are attracted to the area of injury by means of this signaling cascade in order to remodel damaged tissue leading to an increase in cartilage volume (Jo et al., 2017; Jo et al., 2014) another factor also to be considered is the angiogenic capacity of MSCs, the presence of VEG secreted by them directly interferes with the chondrogenic differentiation due to its avascular nature of this tissue (Lee et al., 2012), so the use of platelet-rich plasma has been adopted in conjunction, because in this case it acts as a modulator for this differentiation (Boller & Lopes, 2021; Van Pham et al., 2013), it is important reinforce that when the SVF or micro-fragmented adipose tissue are used as therapy it promote a result not only because of the presence of ADSCs but also because the presence and acting of all components already mentioned. On the other hand, the use of hyaluronic acid and fibrin glue serve as a framework to facilitate the growth of stromal cells and its fixation in the cartilaginous matrix (Pak et al., 2016). The way in which ADSCs were applied to the joint also demonstrated differences, Kim, et al. (2016) compared the injection of ADSCs + PRP versus implantation of ADSCs + fibrin glue at the injury site and concluded that implantation has better results in promoting chondrogenesis (Kim, Kwon, et al., 2015), but we know that if the patient is older than 60 years of age or a lesion is more than 6.0 cm^2 in size, a reduced probability of having reduced improvement (Kim, Choi, & Koh, 2015).

In front of all these information, the knowledge about the use of ADSCs in knee OA is still considered limited in humans, due to several factors that are pointed out as bias in the studies. To understand more about the paracrine effect, that is so discussed, and how it occurs, it is necessary to evaluate the synovial fluid of these patients. Only two of the studies found in this review analyzed the synovial fluid of the knee with treated OA and the maximum follow-up time was 2 years (Bansal et al., 2017; Lapuente et al., 2020). These studies suggested that treatment using SVF + PRP helps to restore synovial fluid properties, restore synovial metabolism, and reduce cartilage pathology (Bansal et al., 2017), in addition to regulating the inflammatory process of OA (Lapuente et al., 2020). Among the aforementioned surveys, several questions still arise: Which growth factors were secreted in the joints by these cells? What specific inflammatory cytokines are altered in synovial fluid? How does the joint environment respond to this therapy? Thus, new studies that have a control group of patients without treatment with ADSCs is of paramount importance to help understand the functioning of this type of cell therapy in humans.

5. Final Considerations

The use of adipose tissue as a source for cell therapy in the knees of humans with OA has shown promise in terms of reducing pain, improving functionality, improving imaging tests, improving the quality of treated cartilage tissue, and in which it was possible evaluate the histology and the amount of cells present in the synovial fluid. However, the mechanisms of ADSCs that promote these results are still being elucidated in the literature for human treatment. The lack of standard protocols to this kind of therapy made that the ADSCS is not the first option in the treatment for cases of knee OA, even with the low occurrence of adverse events. Thus, for ADSCs take their place of importance in the treatment of OA, all the criteria verification proposed by IFATS and ISCT must be made, show, and descript with details in the articles for future studies. In this way, giving greater security to the regenerative medicine community, regarding the real presence of ADSCs in the performed therapies. More comparative studies are needed between extraction methods, number of cells, vehicles, and number of applications, to prove the effectiveness of the methods already published. We still emphasize the importance of a control group in these clinical trials.

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