Intra-Articular Cellular Therapy for Osteoarthritis and Focal Cartilage Defects of the Knee

A Systematic Review of the Literature and Study Quality Analysis

Jorge Chahla, MD*, Nicolas S. Piuzzi, MD*, Justin J. Mitchell, MD, Chase S. Dean, MD, Cecilia Pascual-Garrido, MD, Robert F. LaPrade, MD, PhD, and George F. Muschler, MD

Investigation performed at the Cleveland Clinic, Cleveland, Ohio; The Steadman Clinic, Vail, Colorado; and the Italian Hospital of Buenos Aires, Buenos Aires, Argentina

Background: Intra-articular cellular therapy injections constitute an appealing strategy that may modify the intra-articular milieu or regenerate cartilage in the settings of osteoarthritis and focal cartilage defects. However, little consensus exists regarding the indications for cellular therapies, optimal cell sources, methods of preparation and delivery, or means by which outcomes should be reported.

Methods: We present a systematic review of the current literature regarding the safety and efficacy of cellular therapy delivered by intra-articular injection in the knee that provided a Level of Evidence of III or higher. A total of 420 papers were screened. Methodological quality was assessed using a modified Coleman methodology score.

Results: Only 6 studies (4 Level II and 2 Level III) met the criteria to be included in this review; 3 studies were on treatment of osteoarthritis and 3 were on treatment of focal cartilage defects. These included 4 randomized controlled studies without blinding, 1 prospective cohort study, and 1 retrospective therapeutic case-control study. The studies varied widely with respect to cell sources, cell characterization, adjuvant therapies, and assessment of outcomes. Outcome was reported in a total of 300 knees (124 in the osteoarthritis studies and 176 in the cartilage defect studies). Mean follow-up was 21.0 months (range, 12 to 36 months). All studies reported improved outcomes with intra-articular cellular therapy and no major adverse events. The mean modified Coleman methodology score was 59.1 ± 16 (range, 32 to 82).

Conclusions: The studies of intra-articular cellular therapy injections for osteoarthritis and focal cartilage defects in the human knee suggested positive results with respect to clinical improvement and safety. However, the improvement was modest and a placebo effect cannot be disregarded. The overall quality of the literature was poor, and the methodological quality was fair, even among Level-II and III studies. Effective clinical assessment and optimization of injection therapies will demand greater attention to study methodology, including blinding; standardized quantitative methods for cell harvesting, processing, characterization, and delivery; and standardized reporting of clinical and structural outcomes.

Level of Evidence: Therapeutic Level III. See Instructions for Authors for a complete description of levels of evidence.

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Knee osteoarthritis (OA) is a debilitating disease that is increasing in prevalence[1,2] because of several factors, particularly physical activity leading to intra-articular injury, aging, and rising rates of obesity[2]. Total knee arthroplasty is effective when a trial of nonoperative measures fails; however, functional limitations and the potential need for future revision often necessitate that young and active patients seek other options[3].

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TABLE I The 3 Searches Performed

("stem cells"[MeSH Terms] OR ("stem"[All Fields] AND "cells"[All Fields]) OR "stem cells"[All Fields]) AND ("osteoarthritis, knee"[MeSH Terms] OR ("osteoarthritis"[All Fields] AND "knee"[All Fields]) OR "knee osteoarthritis"[All Fields])
("stem cells"[MeSH Terms] OR ("stem"[All Fields] AND "cells"[All Fields]) OR "stem cells"[All Fields]) AND ("knee"[MeSH Terms] OR "knee"[All Fields] OR "knee joint"[MeSH Terms] OR ("knee"[All Fields] AND "joint"[All Fields]) OR "knee joint"[All Fields]) AND ("cartilage"[MeSH Terms] OR "cartilage"[All Fields])
"cells"[All Fields] AND ("knee"[MeSH Terms] OR "knee"[All Fields] OR "knee joint"[MeSH Terms] OR ("knee"[All Fields] AND "joint"[All Fields]) OR "knee joint"[All Fields]) AND ("cartilage"[MeSH Terms] OR "cartilage"[All Fields])

Interest in minimally invasive methods that may prevent or reverse the progression of cartilage injury or disease has peaked in recent years, particularly for treatment of early OA[4-6] and focal chondral defects[7], which are likely to progress to OA[8]. Numerous injection therapies have been proposed, including hyaluronic acid (HA)[9-11], platelet-rich plasma (PRP)[9,12-14], bone marrow aspirate concentrate[15-17], and other cell-based therapies[18,19].

Recent studies have suggested possible benefits from intra-articular cell injection[19-24]. The purpose of this paper was to provide a systematic review of the current literature and examine the evidence supporting the efficacy and safety of cellular therapy injections for the clinical treatment of OA or focal cartilage defects.

Materials and Methods

Article Identification and Selection

This study was conducted in accordance with the 2009 PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) statement[25]. A systematic review of the literature regarding the treatment of OA and focal cartilage defects in the human knee with intra-articular cellular therapy was performed using the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, PubMed (1990-2014), and MEDLINE (1990-2014). The queries were performed in November 2015 (Table I).

TABLE II Demographic Data of the Included Studies*

Study	Country	LOE	Study Design	Group	Treatment	M:F
Osteoarthritis						
Koh[30]	South Korea	III	Case control			
				Study	PRP + MADNC	8:17
				Control	PRP	8:17
Koh[29]	South Korea	II	RCT			
				Study	HTO + MADNC + PRP	5:16
				Control	HTO + PRP	6:17
Vega[19]	Spain	II	RCT			
				Study	MSC	9:6
				Control	HA	9:6
Focal cartilage defects						
Lee[31]	Singapore	II	Cohort			
				Study	MicroFx + CEACs + HA	16:19
				Control	Periosteal sleeve CEACs	20:15
Saw[32]	Malaysia	II	RCT			
				Study	HA + MBDNC	10:15
				Control	HA	7:17
Wong[33]	Singapore	II	RCT			
				Study	HTO + CEAC + HA	15:13
				Control	HTO + HA	14:14

*The values are given as the mean with or without the standard deviation, and with or without the range in parentheses. LOE = Level of Evidence, FU = follow-up, K-L = Kellgren-Lawrence, RCT = randomized controlled trial, HTO = high tibial osteotomy, FC = femoral condyle, PF = patellofemoral, MicroFx = microfracture, MFC = medial femoral condyle, LFC = lateral femoral condyle, MTP = medial tibial plateau, and LTP = lateral tibial plateau.

Articles presented in the English language that reported clinical outcomes for intra-articular cellular therapy in the human knee with a minimum 12-month follow-up and a Level of Evidence of I, II, or III were considered for inclusion. Cadaveric studies, animal studies, basic science articles, editorials, surveys, special topics, letters to the editor, personal correspondence, studies that did not include the knee, and studies that used cellular therapy for treatment of other non-cartilage pathologic conditions were excluded.

Three investigators (J.C., N.S.P., and C.S.D.) independently reviewed the abstracts of all articles identified in these searches. Full-text articles were reviewed when necessary to confirm that the article satisfied inclusion and exclusion criteria. Reference lists of identified articles were also reviewed to minimize the risk of missing relevant articles.

Data Collection

The Level of Evidence was assigned using classifications specified by Wright et al.[26]. Patient demographics, treatment details, follow-up intervals, and outcome assessments were recorded for each study. Data were recorded into a custom information extraction table[27].

Literature Quality Evaluation

Two reviewers (J.C. and N.S.P.) used a modified version of the Coleman methodology score (mCMS) to assess the quality of methodology in each study[28]. The 2-part mCMS grades cartilage-related studies based on 11 criteria. Part A evaluates the study size; mean follow-up duration; number of different surgical procedures; type of study; and descriptions of the surgical procedure, postoperative rehabilitation, and MRI and histologic outcomes of included subjects. Part B evaluates the outcome criteria, procedure for assessing clinical outcomes, and description of the subject selection process. The maximum score on the mCMS is 100, which indicates that a study largely avoids chance, biases, and confounding factors.

Results

Searches identified 427 individual titles and abstracts (Fig. 1). After removal of 7 duplicates and 386 studies that were eliminated on the basis of the inclusion and exclusion criteria, 34 articles were available for full-text review. After a thorough review of these articles and their citations, a total of 6 studies (4 Level II and 2 Level III) were identified (Table II). Three involved OA[19,29,30] and 3 involved focal cartilage defects[31-33].

The 6 studies included 300 knees (124 with OA and 176 with focal cartilage defects) (Table II). The mean age was 54.85 years (range, 34 to 56 years) for the patients with OA and 45 years (range, 24 to 54 years) for the patients with cartilage defects. The Kellgren-Lawrence (K-L) classification was reported in the OA studies. Only Wong et al. documented the defect size in the studies involving focal cartilage defects[33]. The mean follow-up duration was 21.02 months (range, 12 to 36 months).

TABLE II (*continued*)

Age *(yr)*	FU *(mo)*	Knees	K-L Score	ICRS Grade	Size *(cm^2)*	Location
54.2 ± 9.3	16.4 ± 2.3	25	3.3 ± 0.8			
54.4 ± 11.3	17.2 ± 1.8	25	2.7 ± 0.7			
54.2 ± 2.9	24.2 ± 4.7	21	<4			
52.3 ± 4.9	24.6 ± 6.4	23	<4			
57 ± 9	12	15	2.73 (2-4)			
57 ± 9	12	15	2.8 (2-4)			
						16 FC, 10 PF, 9 multiple
44	24.5	35				
44	24.5	35				
						56% patella, 22% trochlea, 11% MFC, 3% LFC, 5% MTP, 3% LTP
38 ± 7.33	24	25		3-4		
42 ± 5.91	24	25		3-4		
53 (36-54)	24.8 (24-36)	28		3.35 ± 0.6	6 (2.8-12)	MFC
49 (24-54)	24.5 (24-35)	28		3.5 ± 0.5	3.5 (1.5-6.8)	MFC

TABLE III Cell Therapy Descriptions*

Study	Source Site	Collection Technique	Initial Volume	Source	Cell Processing Time	Culture Expansion	Cell Type
Osteoarthritis							
Koh[30]	Adipose: infrapatellar fat pad	Adipose tissue harvest by extension of the skin incision at the arthroscopic lateral portal	Mean weight, 9.4 g (range, 6.9-11.2 g)	Autologous	3-4 hr	No	MADNC
Koh[29]	Adipose: subcutaneous adipose tissue from both buttocks	Tumescent liposuction	120 mL for injection, 20 mL for laboratory analysis	Autologous	NR	No	MADNC
Vega[19]	BMA: iliac crest	Several punctures with 11-G trocar under the iliac spine, aiming toward the posterior sacroiliac joint. Technique involves sudden cortical perforation and repeated aspiration of small BMA volumes (2-4 mL) to minimize contamination with peripheral blood	80 mL	Allogeneic (3 donors)	21-24 days	3rd passage	MSC
Focal cartilage defects							
Lee[31]	BMA: iliac crest	Used Jamshidi BMA needles (11-G, 10 cm). Technique not reported	NR	Autologous	~21 days	1st passage	CEAC
Saw[32]	Peripheral blood progenitor cells (PBPCs)	Automated cell separator (apheresis) by central venous access	NR	Autologous	NR	No	MBDNC
Wong[33]	BMA: contralateral hip	NR	Median aspiration volume, 49 mL (range, 35 to 74 mL)	Autologous	12 days (range, 10-13 days)	1st passage	CEAC

*NR = not reported, and HTO = high tibial osteotomy.

Cellular Therapy

The cell source, collection technique, cell processing, qualitative and quantitative characterization, and delivery method varied widely among the studies (Table III). Details of the tissue collection technique were absent from most reports. Five studies used autologous cells[29-33] and 1 used allogeneic cells[19]. Three studies used freshly isolated tissue-derived nucleated cells, 2 used mixed adipose-derived nucleated cells (MADNCs)[29,30], and 1 used mixed blood-derived nucleated cells (MBDNCs)[32]. Three studies used culture-expanded cells derived from bone marrow aspirate[19,31,33] (CEACs; culture-expanded adherent cells). The cell dose varied from 1.2 to 40 million cells. Qualitative cell characterization of injected cells using surface markers was done in 5 studies[19,29,31-33]. Only Koh et al.[29] assayed the cell population using a colony-forming unit (CFU) assay. Five studies performed 1 cellular therapy injection, supplemented this injection in 3 studies with subsequent doses of PRP or HA[19,29-31,33]. One study[32] performed a

TABLE III (*continued*)

No. of Cells ($\times 10^6$)	No. of CTPs	Injection Site/Technique	Delivery Solution	Qualitative Cell Characterization, CD Markers	Quantitative Cell Assessment, CFU Assay	Successive Injections
1.89 (range, 1.2-2.3)	NR	Classic lateral approach, upper pole of patella, 22-G needle	3.0 mL PRP	NR	NR	3.0 mL PRP on days 7, 14
48.3 (range, NR)	4.11×10^6	Medial, arthroscopic guidance	In 3.0 mL PRP after arthroscopy, before HTO	CD90+, CD105+, CD45−, CD34−, CD14−	CFU >50 cells and adipogenic, osteogenic, and chondrogenic differentiation	None
40	NR	Medial parapatellar	Suspended in Ringer lactate at 5×10^6 cells/mL	Profile of the cultures conformed to the ISCT criteria for MSCs	NR	None
~10	NR	NR	In 2 mL of autologous serum + 2 mL HA	CD90+, CD105+, CD34−, CD14−	NR	2 more doses of 2 mL of HA at weekly intervals for both groups
NR (8 mL)	NR	NR	8 mL MBDNCs + 2 mL HA	CD34+, CD105+	NR	First 5 injections beginning at 1 wk, on a weekly basis. At 6 mo, 3 additional injections weekly
14.6	NR	NR	0.5 to 1 mL of autologous serum + 2 mL HA	CD14−, CD20−, CD34-, CD45−, CD73+, CD90+, CD105+	NR	2 more doses of 2 mL of HA at weekly intervals for both groups

series of 8 cellular therapy injections in the course of treatment.

In addition to cellular therapy, the studies varied widely with respect to adjuvant factors that were included: PRP[29,30], high tibial osteotomy[29,33], HA[31-33], and microfracture[31] (Table II).

Patient-Reported Outcome Measures

Primary outcome measures are summarized in Table IV. Significant improvement in patient-reported outcome measures in the cellular treatment groups were reported in 5 studies: Wong et al.[33], Koh and Choi[30], Koh et al.[29], Vega et al.[19], and Lee et al.[31].

Imaging

The use of imaging also varied widely (Table V). One study[19] in the OA group and 3 in the cartilage defect group[31-33] used MRI (magnetic resonance imaging) for follow-up assessment at 12 to 18 months. Each reported improvement in the treatment group. The Poor Cartilage Index (PCI)[19], the MOCART (Magnetic Resonance Observation of Cartilage Repair Tissue) system[34], and 2 previously unreported subjective systems for MRI assessment[31,32] were used.

TABLE IV Outcome Measures in the Included Studies*

Study	Group	Lysholm		Tegner		VAS	
		Baseline	FU	Baseline	FU	Baseline	FU
Osteoarthritis							
Koh[30]							
	Cell	41.2 ± 12.4	68.1 ± 18.5	1.5 ± 0.5	2.8 ± 1.2	49 ± 12	27 ± 18
	Control	50 ± 11.1	69.4 ± 20.4	2.1 ± 0.8	2.9 + 1.0	39 ± 10	22 ± 17
Koh[29]							
	Cell	55.7 ± 11.5	84.7 ± 16.2			44.3 ± 5.7	10.2 ± 5.7
	Control	56.7 ± 12.2	80.6 ± 13.5			45.4 ± 7.1	16.2 ± 4.6
Vega[19]							
	Cell					54 ± 7	33 ± 6
	Control					64 ± 7	51 ± 8
Focal cartilage defects							
Lee[31]							
	Cell					Graphic	Graphic
	Control					Graphic	Graphic
Saw[32]							
	Cell						
	Control						
Wong[33]							
	Cell	41.9 ± 19.2					
	Control	50.4 ± 23.0					

*The values are given as the mean and the standard deviation. FU = follow-up, VAS = visual analog scale, IKDC = International Knee Documentation Committee, WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index, KOOS = Knee injury and Osteoarthritis Outcome Score, and NR = not reported.

Second-Look Arthroscopy

Second-look arthroscopy was used in 2 studies[29,32]. Koh et al.[29] reported that partial or fibrocartilaginous coverage was achieved in 50% of the treatment group, but in only 10% of the patients in the PRP-only group ($p < 0.001$), at a mean of 20 months after treatment. Saw et al.[32] included cartilage biopsy as well and reported an increase in the HA group on the basis of ICRS (International Cartilage Repair Society) II histological scores ($p = 0.022$).

Safety

Five of the 6 studies reported on adverse events[19,30-33]. There were no major adverse reactions. Among the OA studies, 24 minor events were reported; 23 were reported by Vega et al.[19] and consisted of transient pain, effusion, or inflammation controlled with NSAIDs (nonsteroidal anti-inflammatory drugs). One cartilage defect study reported no complications[31]. Saw et al.[32] reported 85 minor events, most commonly warmth and swelling followed by knee motion difficulty. There was no trend toward greater adverse events in the treatment compared with control groups.

Methodological Quality Assessment

The mean mCMS (and standard deviation) of the 6 studies was 59.1 ± 16 (range, 32 to 82) out of 100 (Table VI). The prospective studies achieved a mean mCMS of 65 (range, 50 to 82).

Discussion

The common thread of the studies reporting intra-articular cellular therapy injections for the treatment of OA and focal cartilage defects in the knee was positive clinical outcomes and no major adverse events. However, the studies were highly heterogeneous and meta-analysis was not feasible. The outcome differences reported between the study and control groups are modest, and randomized but unblinded methodologies do not control for patient or clinician-related bias. As a result, no conclusion can be drawn that current methods of cellular therapy provide generalizable benefit to patients.

The fact that treatment effects were found to be only modest in nature does not diminish the potential value of cellular therapies, however. Treatments with the capacity to modify the intra-articular environment to reduce inflammation, preserve cartilage, or induce cartilage regeneration are of increasing interest because of the rising numbers of patients with diseased cartilage. Both the athletic and aging populations have a strong desire to remain active, enhance or preserve normal knee function, and avoid the expense and risk associated with knee arthroplasty.

As our understanding of the cell populations, biological processes, and environment needed for cartilage homeostasis

TABLE IV (*continued*)

IKDC		WOMAC		Lequesne		KOOS	
Baseline	FU	Baseline	FU	Baseline	FU	Baseline	FU
						NR	81.2 ± 6.9
						NR	74.0 ± 5.7
		41 ± 3	28 ± 5	39 ± 4	30 ± 3		
		45 ± 3	41 ± 6	45 ± 4	42 ± 5		
Graphic	Graphic						
Graphic	Graphic						
46.6 ± 15.8	74.8 ± 12.77						
48.6 + 13.75	71.1 ± 16.49						
33.9 ± 11.4							
36.0 ± 13.7							

and cartilage repair continues to grow, the use and outcomes of intra-articular cellular therapies require further investigation[35]. At present, these interventions have demonstrated only modest clinical improvements, and those findings could be impacted by a placebo effect.

The underlying premise is that the arthritic knee or areas of focal cartilage injury may be deficient in the content of a stem cell or progenitor cell population, and that this deficiency may be mitigated by the harvest and transplantation of cells. There are several possible mechanisms of action for transplanted cells. These mechanisms may theoretically include (1) homing of cells to sites of degenerative or missing cartilage, followed by proliferation and differentiation into functional cartilage or cartilage-like tissue, (2) repopulating of progenitor cell pools on the surface of the synovium or existing cartilage that may subsequently migrate into regions of cartilage damage or augment the ability of existing cartilage to resist degradation, and (3) repopulating of a cell pool that modifies the intra-articular milieu either through cell-cell interactions or through secretion of soluble factors to reduce inflammation and/or activate catabolic agents). These effects could be limited in duration if the transplanted cells survive only a short time in the knee. However, the effects could be long-lasting if the transplanted cells become durable residents in the knee, or if their action induces a durable change in the population of local cells and the intra-articular milieu. Injected cells could induce chemotaxis and migration of other deficient populations of autogenous cells, which may take up longer-term residence in the knee. Alternatively, the injection of cells might induce selective proliferation of local progenitors (i.e., auto-repopulation of otherwise dormant cell populations). To date, the presumed mechanism of action of the cellular therapies has been left largely unaddressed in the clinical literature, including the manuscripts evaluated in this study.

Several additional deficiencies need to be addressed in future investigations, if the field of cellular injection therapy for these and other conditions is to progress: (1) use of a standardized and objective system of nomenclature to describe the cell populations that are administered, (2) objective characterization of the harvest site and methods and of the quality of the starting cell population, (3) quantitative description of the processing methods used and of the effect of cell processing on the concentration and prevalence of the cell population(s) that are presumed to provide therapeutic benefit, (4) quantitative reporting on the composition of the injected cells (concentration, prevalence, and biological potential of various bioactive cell populations), (5) standardized use of patient-reported measures of pain and function before and after treatment, and (6) standardized use of imaging or other means of assessing the structural outcome of injection therapies with respect to cartilage preservation and restoration.

Standardized nomenclature is essential for clarity in scientific communication. *Connective tissue progenitor* (CTP) and *mesenchymal stem cell* (MSC) are terms that should be distinguished. CTPs have been defined as the heterogeneous group of stem cells and progenitor cells present in native tissues that can be activated to proliferate and generate progeny that

TABLE V Structural Assessment*

Study	Group	MRI	Second-Look Arthroscopy	Second-Look Arthroscopy with Biopsy
Osteoarthritis				
Koh[30]		NR	NR	NR
Koh[29]		NR	NR	NR
	Cell	NR	50% even fibrocartilage	NR
	Control	NR	10% even fibrocartilage	NR
Vega[19]				
	Cell	T2 mapping: sign changes. PCI: 0.69, significant ($p < 0.05$) decrease (12 mo)	NR	NR
	Control	T2 mapping: no sign changes. PCI: 0.28, nonsignificant decrease (12 mo)	NR	NR
Focal cartilage defects				
Lee[31]				
	Cell	Good fill and integration. Reduction of marrow edema (12 mo)	NR	NR
	Control	—	NR	NR
Saw[32]				
	Cell	1.5-T MRI, 12-point scoring system: 10.04 ± 1.31 ($p = 0.031$) (18 mo)	NR	Chondral core biopsy, ICRS II score: 1,065 ± 126.75 ($p = 0.022$) (18 mo)
	Control	1.5-T MRI, 12-point scoring system: 8.47 ± 1.75 (18 mo)	NR	ICRS II score: 957.34 ± 126.0
Wong[33]				
	Cell	1.5-T MRI, MOCART: 62.32 ± 17.56 ($p < 0.001$) (1 yr)	NR	NR
	Control	1.5-T MRI, MOCART: 43.21 ± 13.55	NR	NR

*The values are given as the mean and the standard deviation. NR = not reported.

differentiate into 1 or more connective tissue phenotypes (e.g., bone, cartilage, fibrous tissue, fat, muscle)[36-40]. CTPs are rare in native tissues, with a prevalence of between 1 in 2,000 and 1 in 40,000 cells, depending on the tissue. No specific set of markers identifies all CTPs; as a result, the concentration, prevalence, and biological potential of CTPs in a given cell population can only be estimated using in vitro CFU assays. Standardized criteria have recently been incorporated into an ASTM International standard for use with automated systems for image analysis[41]. CTP concentration, prevalence, and biological potential are valuable quality attributes reflecting the regenerative potential of cells that are harvested from tissues and cells that are processed by various means without in vitro culture expansion. These metrics should become standard features in future cellular therapy studies. Of the included studies, only the one by Koh et al.[29] used a CFU assay.

When freshly isolated tissue-derived cells are used, and CTP prevalence and function are not measured on the basis of colony formation, it is most appropriate to define that population of cells as "mixed tissue-derived nucleated cells" (MTDNCs). This designation applies to the cells used in 3 of the 6 included studies[29,30,32].

In distinct contrast to the term *CTP, MSC* was originally defined as denoting a population of purified, homogeneous, culture-expanded cells that retained the capacity to differentiate into multiple tissue types (bone, cartilage, and fat)[42].

However, the term *MSC* has often been misused. The International Society for Cellular Therapy (ISCT) has helped to clarify this point by defining standard criteria that must be present in order to designate a cell population as MSCs: (1) culture-expanded cells that adhere to tissue culture plastic, (2) cells that retain the capability for trilineage differentiation (bone, cartilage, and adipose tissue), (3) cells expressing CD105, CD73, and CD90 (with 95% prevalence), and (4) cells lacking expression of CD45, CD34, CD14 or CD11b, CD79 alpha or CD19, and HLA-DR surface molecules[43]. If these criteria are not met,

TABLE VI Coleman Methodology Scores as Modified by Kon et al.[28]

Section Score (Maximum)	Koh[30]	Koh[29]	Vega[19]	Lee[31]	Saw[32]	Wong[33]	Mean	Std. Dev.
Part A								
Study size (10)	7	7	4	10	7	7	7	1.89
Mean follow-up duration (10)	2	5	2	5	5	5	4	1.54
No. of different surgical procedures included in each reported outcome (10)	4	4	10	0	10	4	5.3	3.93
Type of study (15)	0	15	15	10	15	15	11.7	6.05
Description of surgical procedure given (5)	3	3	5	3	3	3	3.3	0.81
Description of postoperative rehabilitation (5)	5	5	0	5	5	5	4.2	2.04
Inclusion of MRI outcome (10)	0	0	10	5	10	10	5.8	4.91
Inclusion histological outcome (10)	0	0	0	0	10	0	1.7	4.08
Part B								
Outcome criteria (5)	5	5	5	5	5	5	5	0
Procedures for assessing clinical outcomes (10)	3	9	6	4	4	3	4.8	2.31
Description of subject selection process (10)	3	8	8	3	8	8	6.3	2.58
Total for Part A (75)	21	39	46	38	65	49	43	14.51
Total for Part B (25)	11	22	19	12	17	16	16.2	4.16
Total score (100)	32	61	65	50	82	65	59.2	16.82

the term *MSC* should not be used. All 6 of the included studies used the term *MSC* to describe the active component of their cellular therapy, but only 1 of them used the term accurately. Although 2 studies used culture-expanded cells, only the cells studied by Vega et al.[19] satisfied the ISCT criteria for MSCs.

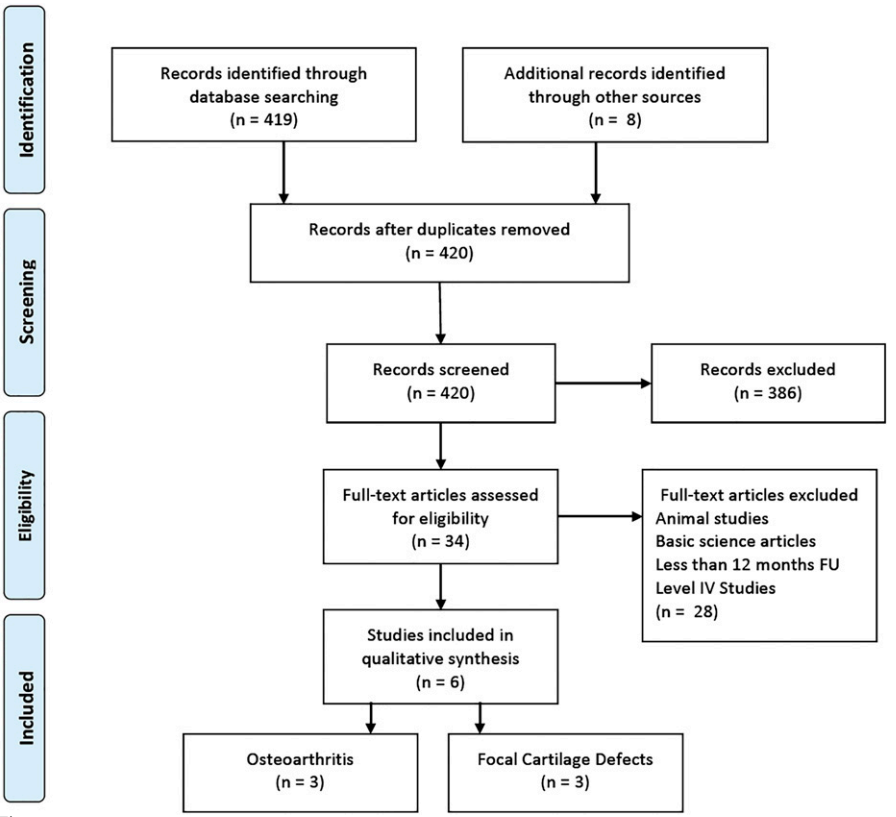

Fig. 1

Flow diagram presenting the systematic review process used in this study. FU = follow-up.

The functional capacity of culture-expanded cell populations is also important, and may not correspond to surface marker expression[44]. As with CTPs, the proliferative capacity of culture-expanded cells and ability of these populations to differentiate into cartilage, bone, and fat under standardized culture conditions are valuable quantitative measures of potency. CFU assays are also appropriate for assessment of the biological potential of culture-expanded populations.

Cell Harvesting and Processing Methods

The cell source (peripheral blood, bone marrow aspirate [BMA], fat), anatomic location (buttocks, retropatellar fat pad), and methods of harvesting (excision, liposuction) and processing (digestion, density separation, in vitro expansion) are essential variables that require specific characterization to ensure reproducibility and systematic refinement in future work.

The technique for obtaining and subsequently processing BMA has a profound effect on the concentration and prevalence of CTPs in the aspirate sample[45]. Of the 3 studies that used BMA as the cell source, only the one by Vega et al.[19] defined both the anatomic site and the technique. The 2 studies using adipose tissue[29,30] both processed cells with enzymatic digestion and density separation with a centrifuge, but provided no data with respect to CTP yield or the biological performance of these processed cells.

Quantitative reporting of the composition of the injected cells requires characterization. This includes the number of nucleated cells, erythrocytes, and platelets, as well as the differential count of the nucleated cells. Estimates of CTP prevalence and biological performance based on CFU assays should be required for populations of freshly isolated cells. Characterization of cells with respect to cell surface markers (e.g., by flow cytometry) should be required for culture-expanded populations. Flow cytometry is not inappropriate for characterization of freshly isolated cell populations, but it has little value in estimating the prevalence of CTPs among these mixed cell populations. The heterogeneity of colony-founding CTPs with respect to CD markers and the very low initial prevalence of CTPs in the starting population of freshly isolated cells (often <1 in 10,000) leave CTPs undetectable by traditional flow cytometry.

Safety concerns are particularly important when considering cellular therapies, especially for non-life-threatening disorders. The risks involved in short-term processing are primarily the risk of compromised sterile technique or cell toxicity during processing. Culture expansion methods introduce the additional risk of inadvertent selection of clones with undesirable epigenetic or genetic changes[46]. This review did not reveal any safety concerns, particularly in the setting of autogenous cell transplantation. Only minor events were reported. This observation is comparable with previous reports of cell-based treatments in the orthopaedic literature[47,48].

In conclusion, the efficacy of cellular therapy injections has not yet been established. The value and effective use of cell therapy in orthopaedics remain unclear largely because of the absence of (1) rigorous blinded clinical trials, (2) standardized use of nomenclature to define cell populations, and (3) quantitative metrics to define cell populations and clinical and structural outcomes[49]. Although many of the studies reported here were randomized, patients had not been blinded. Because cellular therapy carries a high level of expectation for possible benefits, it can constitute a strong source of bias in enrollment and in perception of patient-reported outcome[50]. Future clinical trials must overcome the abovementioned deficiencies[50,51]. ■

Jorge Chahla, MD[1]
Nicolas S. Piuzzi, MD[2]
Justin J. Mitchell, MD[1]
Chase S. Dean, MD[1]
Cecilia Pascual-Garrido, MD[3]
Robert F. LaPrade, MD, PhD[1,4]
George F. Muschler, MD[5]

[1]Steadman Philippon Research lnstitute, Vail, Colorado

[2]Institute of Orthopedics, "Carlos E. Ottolenghi" Italian Hospital of Buenos Aires, Buenos Aires, Argentina

[3]University of Colorado, Aurora, Colorado

[4]The Steadman Clinic, Vail, Colorado

[5]Department of Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio

E-mail address for J. Chahla: jchahla@sprivail.org
E-mail address for N.S. Piuzzi: nicolas.piuzzi@hospitalitaliano.org.ar
E-mail address for J.J. Mitchell: jmitchell@thesteadmanclinic.com
E-mail address for C.S. Dean: cdean@sprivail.org
E-mail address for C. Pascual-Garrido: cecilia.pascualgarrido@gmail.com
E-mail address for R.F. LaPrade: drlaprade@sprivail.org
E-mail address for G.F. Muschler: muschlg@ccf.org

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