European Review for Medical and Pharmacological Sciences 2021; 25: 7754-7764

Stem cells application in meniscal tears: a systematic review of pre-clinical and clinical evidence

G. RINONAPOLI[1], P. GREGORI[1], B. DI MATTEO[3,5], L. IMPIERI[2], P. CECCARINI[1], F. MANFREDA[1], G. CAMPOFREDA[3,4], A. CARAFFA[1]

[1]Department of Orthopaedic and Trauma Surgery, Perugia Hospital, Perugia, Italy
[2]Residency Program in Orthopedics and Traumatology, University of Milan, Milan, Italy
[3]Humanitas University, Department of Biomedical Sciences, Milan, Italy
[4]IRCCS Humanitas Research Center, Rozzano (MI), Italy
[5]Sechenov First Moscow State Medical University (Sechenov University), Department of Traumatology, Orthopaedics and Disaster Surgery, Moscow, Russia

Abstract. – OBJECTIVE: Conservative and surgical treatments for meniscal lesions are various and this field of orthopedic surgery is in continuous development. Stem cells represent one of the current options to stimulate meniscal healing. The present systematic review aimed at summarizing the state of art in the application of stem cells for the treatment of meniscal damage both at pre-clinical and clinical level.

MATERIALS AND METHODS: The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were followed to perform this systematic review. A systematic search was performed using the PubMed (MEDLINE), EMBASE and Cochrane Library databases. All the studies dealing with the application of stem cells as a treatment for meniscal tears were pooled, data were extracted and analyzed. The studies were divided into two groups (pre-clinical and clinical), and then, discussed independently.

RESULTS: A total of 18 studies were included. Thirteen were classified as "pre-clinical" and five as "clinical". The most commonly used cells were mesenchymal stem cells (MSC), derived from bone marrow (BMMSC), synovial tissue (SMSC), or adipose tissue (ADSC). Follow-ups ranged from 2 to 16 weeks for the pre-clinical studies and from 3 to 24 months for the clinical studies. All studies documented good results in terms of laboratory markers/scores, clinical and radiologic evaluation.

CONCLUSIONS: Based on the currently available data, it is not possible to establish the best cell source or delivery method for the treatment of meniscal injuries. Bone Marrow derived stem cells delivered through injection represent the most studied approach, with the most promising results. However, the full impact of these therapies through their different sub-type of stem cells and implantation techniques still needs to be critically analyzed through larger randomized controlled trials with longer follow-up.

Key Words:
Meniscal tears, Stem cells, Stem cells injections, Conservative therapy, Meniscal lesions, Clinical benefits.

Introduction

The meniscus has a complex anatomy and serves a variety of biomechanical functions, such as load bearing, shock absorption, guiding rotation and stabilizing translation between the tibia and femur[1]. Meniscal lesions, the most common intra-articular knee injury, represent the most frequent cause of surgical procedures performed by orthopedic surgeons, especially following sports injuries[2-4]. In the United States, the average annual incidence of meniscal lesions has been reported to be 66 per 100,000 inhabitants, 61 of which undergo a meniscectomy[5]. Studies[6] investigating biomechanical effects of the total meniscus removal found a 50% decreased load transmission in the total contact area, resulting in a 335% increase in the local contact load. In addition, partial meniscectomy (16 to 34%) has shown to cause an almost quadrupled contact force on the articular cartilage. Some studies[7-10] reported an increased risk of developing tibio-femoral osteoarthritis, a painful chronic disease, in patients treated with partial or total meniscectomy, with a significant burden on healthcare costs worldwide. Due to its anatomy and vascularity, the meniscus has limited healing capacities, especially in the central two-thirds avascular zone, while meniscal tears in more peripheral vascular

Corresponding Author: Pietro Gregori, MD; e-mail: pietroflaviogregori@gmail.com

zone are more easily repairable[11]. The current trend in treating meniscal lesions is to maintain the tissue intact whenever possible in order to avoid long term complications[12]. However, restoring the meniscus both anatomically and functionally continues to be challenging[13]. Orthopedic surgeons have shifted their goal from resection to preservation, repair, and regeneration of the meniscus[14], leading meniscal suture to become the gold standard for treating this type of lesions. On the other hand, tears of the avascular zone, including radial tears, are not expected to heal and have been mostly treated by partial meniscectomy rather than suture[15]. Several therapeutic options have been proposed to reconstruct the meniscus using autografts: fat pad[16], tendon[17], periosteum[18], perichondrium[19]. They often ended up as being a non-valid option due to their different biomechanical characteristics[20]. Recently, tissue engineering using stem cells, biological scaffolds and growth factors have gained increasing attention as potential regenerative treatments in the orthopedic field[13]. Most of these approaches are still in the preclinical phases but are likely to progress to the clinical stage in the future[21]. Different types of cells have been considered as a treatment for regenerating or repairing the meniscal tissue: meniscal cells[22], chondrocytes and mesenchymal stem cells (MSCs) derived from bone marrow[23], fat tissue[24], scaffolds for cartilage tissue engineering[24] or synovium[25]. Among these, stem cells, as well as their various applications, represent the most studied option. However, there is still no consensus regarding the best cell resource or method of delivery due to the low number of studies available on this topic. The present systematic review aimed at delineating the state of art on the use of stem cells as a treatment option for meniscal tears, to identify the cell types, sources and delivery modalities currently adopted, and to describe the histological, radiologic and clinical outcomes following application of stem cells both at pre-clinical and clinical level.

Materials and Methods

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines have been used to carry out this systematic review[26].

Eligibility Criteria

Studies written in English, Italian, French, Spanish and German were eligible for inclusion. Only peer-reviewed journals were considered, and laboratory *in vivo* or *in vitro* studies, randomized controlled trials (RCTs), prospective and retrospective comparative studies and case series were included, all dealing with the use of stem cells as a treatment for meniscal tears. Exclusion criteria were: reviews of the literature, expert opinions, and studies that did not evaluate the use stem cells for meniscal application.

Information Sources and Search

An electronic systematic search of CINAHL, EMBASE, PubMed and the Cochrane Central Registry of Controlled Trials was carried out by two reviewers (P.G. and L.I.), in order to identify eligible studies. The search was executed on 10 June 2021. The utilized search strings were: ((Stem Cells [MeSH Terms]) AND Meniscal tears MeSH Terms]); (((meniscal tears [MeSH Terms]) AND Regeneration [MeSH Terms]) AND Stem Cells) AND outcomes.

Study Selection

Once the duplicates had been removed, relevant articles from the electronic search were retrieved in full text and evaluated. A manual search of the bibliography of each published study was performed, in order to find relevant articles that could potentially have been missed. Reviews, systematic reviews and meta-analyses were also retrieved and evaluated, in order to broaden the search to include studies that might have been missed. The remaining articles were analyzed by two reviewers (P.G. and G.R.), to exclude studies not fulfilling the eligibility criteria. The reviewers were not blinded to the authors, year and journal of publication. Clinical studies eligible for inclusion were categorized by study type, according to the Oxford Centre for Evidence-Based Medicine (www.cebm.net). The following categories were utilized: case report (CR), randomized controlled trial (RCT) and case series (CS). Since a score to evaluate such a heterogeneous cohort of studies could not be found, the minimum quality of the study, hence inclusion, was discussed in the group. After a first selection by 6 authors (P.G., L.I., B.D.M, F.M, P.C., G.C.), studies were presented to the two senior authors (GR and AC) who performed a final assessment, which included a discussion to reach consensus in case of disagreement.

Data Collection Process

Two assessors independently extracted data from eligible studies using a data extraction form that was predefined according to the protocol. For each study, we extracted the epidemiological characteristics of participants, and assessment

of results. Data were analyzed using R software (2020; R Core Team).

Data Extraction

All the included studies that met inclusion criteria were analyzed and following data were extracted and summarized in tables using Microsoft Excel: study type, year of publication, type of cells used, number of patients, follow up, animal model, type of defect, number of patients, procedures, measures and results. Studies were divided into different categories (Pre-clinical/laboratory and clinical).

Results

A total of 34 studies were found in the electronic search; of these, 16 were eligible for inclusion in this systematic review. Two more studies were identified as relevant through the manual search and were included. A total of 18 studies were thus included, all were *in vitro*/*in vivo*, case series, case control or case report. The study selection process is shown in Figure 1. Pre-clinical studies details are summarized in Table I, clinical studies details are summarized in Table II.

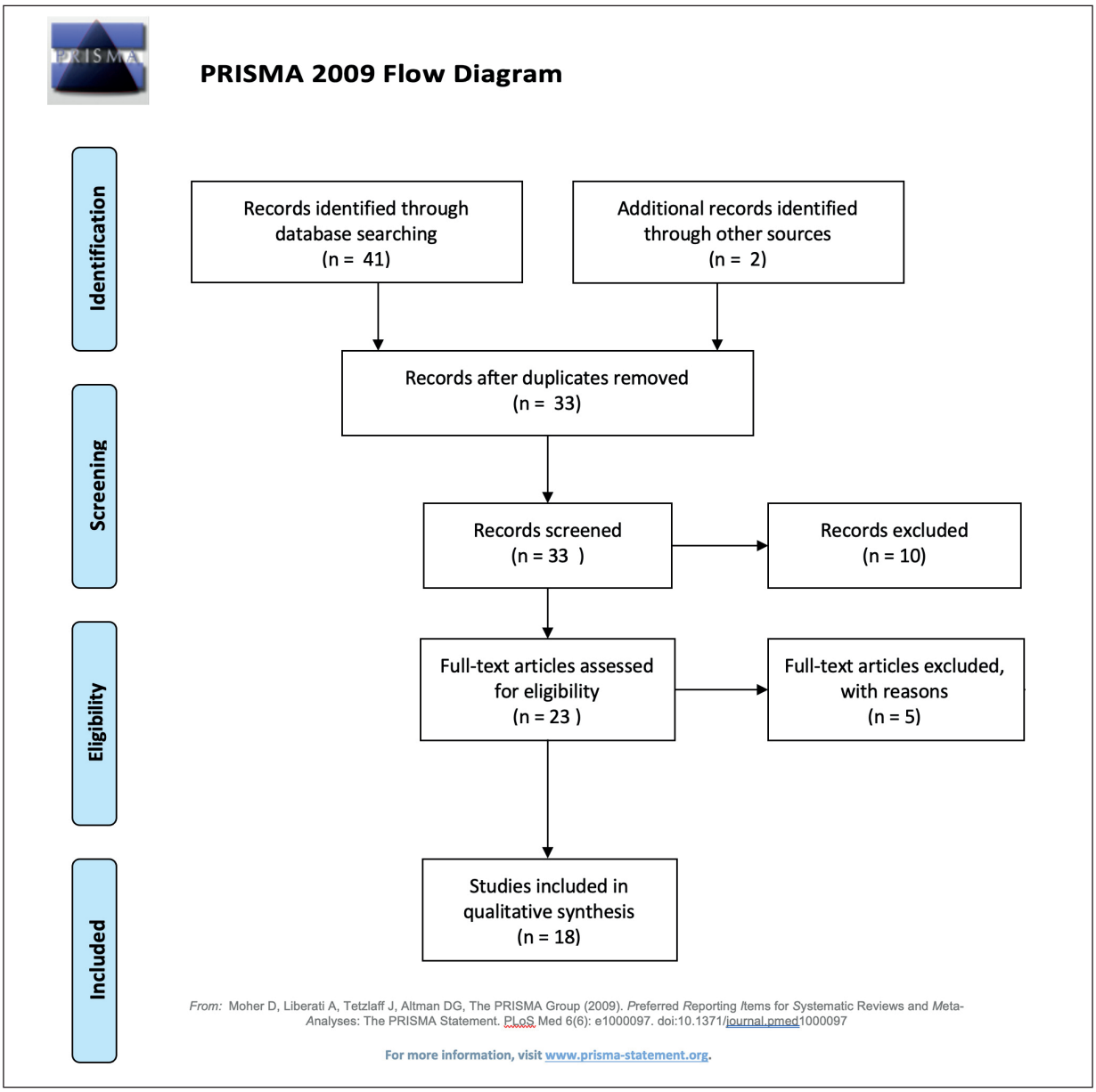

Figure 1. Prisma flowchart of the study selection process.

Table I. Pre-clinical study details.

Study	Year	Type of cells	Type of study	Follow-up
Horie et al[27]	2009	SMSCs (n=14) *vs.* BMMSCs (n=9)	*vitro* + *vivo*	The whole medial meniscus was collected at 2, 4, 8, and 12 weeks after MSCs injection
Zhang et al[32]	2009	BMMSC with transfection of hIGF-1/30 × 106/mL/Calcium alginate gel into defect	*vitro* + *vivo*	16 weeks
Shen et al[33]	2013	MeSCs	*vitro* + *vivo*	4, 8, and 12 weeks
Moriguchi et al[25]	2013	SMSC/0.2 × 106 cells—3 weeks culture/3D matrix construct (TEC)	*vitro* + *vivo*	6 months
Okuno et al[34]	2014	SMSCs syngenic *vs.* allogenic	*vitro* + *vivo*	2,4,8 weeks
Ding et al[29]	2015	MSCs *vs.* BMMSCs (*in vivo*: The stem cells with the density of 6 × 104 cells/0.1 ml were mixed with 0.5 ml of Matrigel and cultured with DMEM-10% FBS at 37 °C and 5% CO_2)	*vitro* + *ex vivo* + *in vivo*	*in vitro* 3 week Ex vivo 6 weeks, *in vivo* 3 weeks.
Ozeki et al[35]	2015	SMSCs	*vitro* + *vivo*	2,4,8 weeks
Gonzalez et al[28]	2015	BMSc *vs.* ADSCs (A collagen repair patchl was used as a scaffold to evaluate regeneration of meniscal tissues)	*vitro* + *vivo*	—
Desando et al[36]	2016	Bone marrows concentrate or BMSCs/BMC: 39 × 106 BMSCs: 6 × 106/ Arthrotomy Bone marrow or BMMSC in HA mesh	*vitro* + *vivo*	12 weeks
Zellner et al[30]	2017	BMSc *vs.* MMSc (collagen hyaluronic scaffolds)	*vitro* + *vivo*	6 or 12 weeks
Moradi et al[37]	2017	ADSCs *vs.* ADSCs+ACs (Polyvinyl alcohol/Chitosan (PVA/Ch) scaffold)	*vitro* + *vivo*	7 months
Kondo et al[38]	2017	SMSCs/0.25 × 106/Aggregates	*vitro* + *vivo*	8 and 16 weeks
Takata et al[39]	2020	ADSCs sheet and CD sheet (Cell death (CD) sheets were created by killing ADSCs by freezing to investigate the need for viable ADSCs in ADSC sheets	*vitro* + *vivo*	4 and 12 weeks

Note: *represents a comparison with mild conditions, $p<0.050$; #represents a comparison with moderate conditions, $p<0.050$; represents a comparison with the initial onset type, $p<0.050$; &represents a comparison with the chronic recurrence type, $p<0.050$; ^represents a comparison with the chronic recurrence type, $p<0.050$.

Pre-Clinical Studies

A total of 13 studies were classified as pre-clinical. All of them were *in vitro* or *in vivo/ex vivo*. The main analyzed stem cells in the studies were mesenchymal stem cells (MSC), derived from bone marrow (BMMSC), synovial tissue (SMSC), or adipose tissue (ADSC).

The selected studies have been conducted on six different species of animals: rabbits (5 studies), rats (3 studies), goats (2 studies), pigs (1

Table II. Clinical study details.

Study	Year	Type of cells	Type of study	Follow-up
Centeno et al[40]	2008	Iliac crest BMAC	Case control	3 months
Vangsness et al[31]	2014	Allogeneic MSCs derived from BMAC	Randomized control trial	24 moths
Pak et al[41]	2014	Abdominal liposuction	Case control	18 months
Onoi et al[42]	2019	Liposuction from thigh	Case report	6 months
Sekiya et al[43]	2019	Arthroscopically harvested Synovial Tissue	Case series	24 moths

study), horses (1 study), monkeys (1 study). Follow-up ranged from 2 weeks to 16 weeks. The most common defect model analyzed was massive tear of medial meniscus. Seven studies delivered the stem cells through injection, three studies used the applications of scaffolds while just one study used removable sheets. A synopsis of all the pre-clinical studies with their major findings is reported in Table III.

Clinical Studies

A total of 5 studies were classified as pre-clinical. Two case controls, one case series, one case report and one randomized control trial. The main analyzed stem cells in the studies were mesenchymal stem cells (MSC), derived from bone marrow (BMMSC) and synovial tissue (SMSC), or adipose-derived stem cell (ADSC). The harvest sites were: iliac crest, abdominal adipose tissue, thigh adipose tissue and arthroscopically harvested synovial tissue. Follow-up ranged from 3 months to 24 months. The most common defect model analyzed was tear of medial meniscus. Four studies delivered stem cells through a perilesional injection, in one study cells being delivered through an arthroscopic transplantation of autologous synovial MSC suspension. A synopsis of all the clinical studies with their major findings is reported in Table IV.

Discussion

Treatment goals and techniques for meniscal tears have completely changed over the last 20 years. Although surgical technologies improved over the years, several authors[7,8] demonstrated a need for an efficient conservative treatment that could prevent the increased risk of developing tibiofemoral osteoarthritis in patients treated with partial or total meniscectomy. The use of stem cell therapies, through various delivery systems, has been selected by several clinicians and laboratory groups as the most promising therapy to invest on in terms of research[23,25]. Encouraging results have been collected by *in vitro/in vivo* studies, but human studies thus far have been limited. This systematic review aimed to delineate a univocal subtype of stem cells or delivery system as the more promising to pursue. Overall, a total of 18 studies, either pre-clinical or clinical, have been found through literature search. All of them, focusing on the use of Mesenchymal Stem Cells (MSC) or adipose-derived stem cells (ADSC), showed good results. Quantitative analysis of the results could not have been calculated for pre-clinical studies due to the different measures used by the authors. Moreover, imaging evaluations and clinical scores have shown significant improvement for patients of the clinical studies. All studies used MRIs from 3 up to 24 months from surgery to assess the results of the treatments on the lesions and calculate the meniscal volume. All studies considered either the reduction of the lesion size, through an imaging measurement, or an increase of the meniscal volume, as a sign of efficacy of the treatment. This evaluation is fundamental since it is the only compatible, in terms of costs, with the eventual future use of this treatment in the clinical routine. Indeed, only one study evaluated these "repair parameters" through a second-look arthroscopy. Even if evidence is too low to draw reliable conclusions about the efficacy of these treatments, our study underlines the potential of stem cells. Horie et al[27] found no macroscopically remarkable differences between the synovium-MSC injection group and the bone marrow-MSC injection group. After 12 weeks of SyMSC, the renewed menisci were LacZ positive, showed their features by transmission electron microscopy and were able to produce type 2 collagen. Similar findings were reported by Gonzalez et al[28], who found no differences between the two populations of cells. Both types of MSCs had universal stem cell characteristics (CD90 and CD105 but not CD73). For *in vivo* testing, at 12 months after treatment, treated defects were regenerated with fibrocartilaginous tissue ($p<0.001$), whereas untreated defects were only partially repaired or not repaired at all. These results are in contrast with Ding et al[29], who, in 2015, compared two subtypes of stem cells and found significant differences between them. In their study, they shared different stem cell marker expression as strol-1, CD90 and CD44, Nanog, nucleostemin, SSEA-4. After all, there were several differences between MMSCs and BMSCs. The former had much smaller colonies than the latter, and these colonies grew even faster than MMSCs. Furthermore, CD34 was more expressed in BMSCs than in MMSCs. At last, in *in vivo* and *in vitro* analysis, MMSCs had a strong predisposition to chondrogenic differentiation (46% *vs.* 32%) and BMSCs had a greater osteogenic potential (44% *vs.* 32%). After a 6 weeks culture, the wound area in rabbit meniscus healed almost entirely by MMSCs treatment, whereas for BMSCs treatment, only 80% was healed.

Table III. Pre- clinical rates and specifics

Study	Animal defect/ model	Procedures	Control	N° of cells	Measures	Results
Horie et al[27]	Rats with massive meniscal damage (anterior half of the mm was removed)	Injection	Same volume of PBS was injected into the other knee	5x × 10^6	1) *in vitro* Differentiation Assay for adipogenitc, chondrogenic and osteogenic differentiation 2) flow cytometry for CD90, CD29 CD11b and CD45. 3) *in vivo* Bioluminescent Imaging 4) Quantitative Real-Time PCR to detect any cell in other organs	The two injection groups of bone marrow-MSC and synovium-MSC showed no significant differences. After 12 weeks of SyMSC, the renewed menisci were LacZ positive, showed their aspects by transmission electron microscopy and were able to produce type 2 collagen. Real-time PCR detected LacZ gene of MSCs in no other organ except the synovium. In in-vivo luminescence analysis were remarked, for three days, increasing photons in the resected knee – meniscus, then an appreciable decrease with no the detection in all other organs.
Zhang et al[32]	Goat/full thickness defect in medial meniscus anterior horn	Injection	No treatment	30 × 10^6	Gross morphology Histology	BMMSC w/hIGF-1 group had better repair tissue without clear margin. Large number of well aligned cells within repair defect. TEM showed round oval like chondrocyte like cells. MRI: smooth continuous anterior horn Higher GAG content to control
Shen et al[33]	NZW Rabbits. The anterior half of medial meniscus was removed to create a defect	Injection	Same volume of PBS was injected into the left knee (the control group) as control.	6 × 10^6	1) *in vitro* Differentiation Assay for adipogenitc, chondrogenic and ostheogenic differentiatio 2) Flow cytometry analysis of MHC-II expression by MeSCs to evaluate the immunogenicity of rabbit MeSCs 3) Cell labeling and detection Prestained with fluorescent dye DiI/CFDA (carboxy-fluorescein diacetate). 4) Histology evaluation was performed using the modified Mankin's score. 5) Radiographic evaluation The X-ray photograph (A-P) of knee joint (containing femur and tibia) 6)Immunohistochemistry for collagen II 7) TEM to assess cell shape within the regenerated meniscus. 8) Real-time PCR analysis of the expression of the two genes collagen type II and biglycan (BGN) 9) The compressive mechanical properties of meniscus was performed (n = 5 for the MeSC-treated group and n = 5 for the control group) using an Instron tension/ compression system	Within the rabbit meniscus tissue were isolated and then identified the only cell subpopulation with typical features of MeSCs (Mesenchymal Stem Cells). MLC suggest that immune response in *in vitro* analysis is suppressed by MeSCs and that they are considered nonimmunogenic; intra-articular injection of allogenus MeSCs was able to stimulate the meniscus regeneration *in vivo* with no immunological rejection and to protect the joint about its surface and the space width, in a rabbit knee joint.
Moriguchi et al[25]	Pig/4 mm cylindrical defect in medial meniscus	Aggregates	No treatment	0.2 × 10^6	Gross morphology Histology	TEC implanted defects showed fibrocartilaginous repair and integration compared to control. Histo: cartilage like cells with nuclei in lacuna
Okuno et al[34]	The anterior half of medial meniscus in both knees of rats was removed	Injection	F344 (syngenic) *vs.* Lewis (minor mismatched transplantation) *vs.* ACI (major mismatched transplantation)	5 × 10^6	1) *in vitro* Differentiation Assay for adipogenitc, chondrogenic and osteogenic differentiation 2) The removed medial menisci were photographed, and their sizes were quantified with Image J software 3) Histology Regenerated meniscus tissue 4) Immunohistochemistry for collagen I and II, ED1 and CD8	The area of the renewed meniscus in the F344 group was remarkably larger than that in the ACI group at four weeks. Histological score in the Lewis and F344 groups, at eight weeks, was remarkably better than in the ACI group. At 1 week, DiI labeled cells were detected in the knee joint in the F334 group but not in ACI group; in the F334 group the number of macrophages and CD8 T cells around meniscus defects was considerably lower than in the ACI group. Meniscus regeneration was promoted better by syngeneic and minor mismatched transplantation of synovial MSCs than major mismatched transplantation.

Continued

Table III (continued). Pre- clinical rates and specifics

Study	Animal defect/ model	Procedures	Control	N° of cells	Measures	Results
Ding et al[29]	N.Z. White rabbits in total (4 for *in vivo*). Wound with 1 mm diameter was created in the center of each meniscus by a biopsy punc.	Either BMSCs or MMSCs at passage 2 were seeded in the defects, culturing with 10% FBS-DMEM for 6 weeks. For *in vivo* wound in the back of 4 rabbits.	—	6×10^4/0.1 mL	1) Immunocytochemistry for expression of the stem cell markers. 2) Multi-differentiation potential of MMSCs and BMSCs *in vitro* were tested with Oil Red O for adipogenesis, Safranin O for chondro- genesis, or Alizarin Red S for osteogenesis. 4) The specific gene expression of differentiated MMSCs and BMSCs were determined using qRT-PCR. 5) The chondrogenesis differentiation of BMSCs and MMSCs on wounded rabbit meniscus was tested by histochemical staining. 6) The multi-differentiation potentials of two kinds of MSCs *in vivo* were tested by immunostaining on tissue sections of nude rats after implantation.	They shared different stem cell markers expression as strol-1, CD90 and CD44, Nanog, nucleostemin, SSEA-4. After all, there were several differences between MMSCs and BMSCs. The former had much smaller colonies than the latter, which colonies grew even faster than MMSCs. Furthermore, CD34 was more expressed in BMSCs than in MMSCs. At last, in in-vivo and *in vitro* analysis, MMSCs had a strong predisposition to chondrogenic differentiation (46% *vs.* 32%) and BMSCs had a greater osteogenic potential (44% *vs.* 32%). After a 6 weeks culture, the wound area in rabbit meniscus was healed almost entirely by MMSCs treatment, whereas for BMSCs treatment, only 80% was healed.
Ozeki et al[35]	Removed the anterior half of the medial meniscus	Autologous Achille's Tendon immersed in the SyMSCs suspension + injection	Achilles tendon graft surgery without synovial MSCs (Tendon group; n 5) or only meniscectomy (Untreated group; n 5).	One million synovial MSCs	1) Macroscopic pictures Quantification 2) histological examination The regenerated meniscus was evaluated using the quantitative score based on the Pauli's score 3) Immunohistochemistry for collagen II 4) i*n vivo* Bioluminescent Imaging 5) Fluorescent Macroscopic and Microscopic Examination. Detection of GFP in the GFP1 tendon graft into the wild type rat, or wild type tendon graft into the GFP1 rat 6) Flow Cytometry for CD90, CD45, CD29, CD31	Tendon grafts increased meniscus size irrespective of synovial MSCs. Histological scores for regenerated menisci were better in the tendon 1 MSC group than in the other two groups at 4 and 8 weeks. Both macroscopic and histological scores for articular cartilage were significantly better in the tendon 1 MSC group at 8 weeks. Implanted synovial MSCs survived around the grafted tendon and native meniscus integration site by cell tracking assays with luciferase1, LacZ1, DiI1, and/or GFP1 synovial MSCs and/or GFP1 tendons. Flow cytometric analysis showed that transplanted synovial MSCs retained their MSC properties at 7 days and host synovial tissue also contained cells with MSC characteristics. Synovial MSCs pro- moted meniscus regeneration augmented by autologous Achilles tendon grafts and prevented cartilage degeneration in rats.
Gonzalez et al[28]	Female Hispano-Breton horses, A lesion (10 mm in length and 6 mm in depth) was created in the cranial part N of the medial meniscus of both stifle joints of each horse.	Scaffold	The 3 horses of one group received treatment with BM-MSCs, whereas the 3 horses of the other group received treatment with AT-MSCs. One stifle joint in each horse was arbitrarily selected for treatment with cultured MSCs loaded onto a collagen scaffold; the contralateral stifle joint in each horse was not treated with MSCs and served as a cell-free control joint.	—	1) MSC characterization (CD90, CD73 and CD105) 2) Multidifferentiation potential of both cells (adipo *vs.* chondro *vs.* ostheo) 3) Cell proliferation in the scaffolds was evaluated by use of DNA quantification and a cell viability assay. 4) macroscopic assesment 12 month after surgery (Quantification of re- generated meniscus was performed by measuring the amount of safranin O fast green staining)	Both types of MSCs had universal stem cell characteristics (CD90 and CD105 but not CD73). For *in vivo* testing, at 12 months after treatment, treated defects were regenerated with fibrocartilaginous tissue (P<0,001), whereas untreated defects were partially repaired or not repaired.

Continued

Table III (continued). Pre- clinical rates and specifics

Study	Animal defect/ model	Procedures	Control	N° of cells	Measures	Results
Desando et al[36]	Sheep/Unilateral medial meniscectomy	Arthrotomy Bone marrow or BMMSC in HA mesh	No treatment	BMC:39 × 10^6 BMSCs: 6 × 10^6	Gross morphology, Microtomography, Histo-Immunohistology	Meniscal tissue regeneration greatest in BMC + HA group. Both BMC and BMSCs group showed good cell density and proteoglycan content compared to control. BMC+ HA group had higher expression of Col II than I compared to BMSCs group
Zellner et al[30]	NZW Rabbits with lateral meniscus was luxated anteriorly and avascular meniscal defects made by using a 2-mm punch device Human meniscal specimens were obtained.	Scaffold	On the one side, the punch defect was treated by an autologous MSC matrix composite and, on the contra-lateral knee, the punch defect in the lateral meniscus was filled with an autologous meniscal cell matrix composite. The animals were sacrificed at 6 or 12 weeks.	1.0 × 10^6 cells into the composite scaffold	Gross joint morphology and OARSI grade were assessed, and menisci were harvested for macroscopic, histological, and immunohistochemical evaluation using a validated meniscus scoring system (Subgroups in macroscopical assessment were "stability" and "defect filling with repair tissue"; for histological analysis the "quality of the surface area", "integration", "cellularity", "cell morphology"; and for immunohistochemical characterization the "expression of proteoglycan and moderate collagen type II in the repair tissue"). In conjunction, human meniscal cells isolated from non-repairable bucket handle tears and human MSCs were expanded and, using the pellet culture model, assessed for their meniscus-like potential in a translational setting through collagen type I and II immunostaining, collagen type II enzyme-linked immunosorbent assay (ELISA), and gene expression analysis.	Osteoarthritic defects came out in all knees, after the resection of the medial menisci (average OARSI grade 3.1). Anyhow, meniscus punch defects were mostly repaired using either MSCs or meniscal cells. The new tissue, histologically, was meniscus-like with classic pericellular meniscal cavities, a significant amount of extracellular matrix and a low cell density. Immunostaining for collagen II was considerably positive. Characteristic radially orientated collagen fibers could be noticed in the architecture of the reconstructed meniscus. Gross joint assessment demonstrated donor site morbidity for meniscal cell treatment. The gene expression and production of collagen type II in human MSCs was definitely higher than in meniscal cells ($p<0.05$).
Moradi et al[37]	New Zealand rabbits underwent unilateral total medial meniscectomy.	Scaffold	Positive control group (n=3): rabbits received no surgical intervention. Experimental groups including: AC/scaffold group, ASC/scaffold group, AC-ASC/scaffold group (7 animals in each group) and cell-free scaffold group (n=5).	—	Fourier Transforms Infrared (FTIR) Spectroscopy, Measurement, Degradation Rate, Scanning Electron Microscopy (SEM), Cell Viability, RT-PCR was performed to assess expression of collagen I, II and aggrecan, Macroscopic evaluation, Histological and IFC analysis for neomeniscus tissue, Evaluation of femoral condyle and tibial plateau cartilage	Better results in immunoflorescent, histologic and post-implantation, for regenerated meniscus in AC/ scaffold group, than in AC-ASC/scaffold and ASC/ scaffold groups, in cell-free scaffold group there was no meniscus regeneration. In ASC/scaffold group there were the worst degenerative defects, followed by the cell-free scaffold group; histologically, the best score was observed in AC/scaffold group.
Kondo et al[38]	Monkey/Anterior horn of medial meniscus Partial Meniscectomy	Aggregates	No Treatment	0.25 × 10^6	Macro and Histology analysis, MRI	Macro: Regeneration in control and MSCs groups with MSC showed larger medial meniscus at 8 and 16 weeks. Histo: Safranin-O slight staining at 8 weeks, positive at 16 weeks. No staining in control MRI: MSC groups closer resembled intact menisci compared to control.
Takata et al[39]	42 mature female Japanese white rabbits, The anterior half of the medial meniscus was removed to create a defect.	Sheet	The contralateral limb was closed 15 without trans-plantation following meniscal removal	1 Sheet	The meniscal tissue area, transverse diameter on the inside of the medial collateral ligament, and histological score.	At 4 and 12 weeks, the area and transverse diameter of regenerated tissues were bigger than in the ADSC sheet group than in control group. Moreover, the histological score in the ADSC sheet group was definitely higher than the control group at four weeks (p = 0.02) and even more high than that in CD sheet group (ADSC = 12.5, p= 0.009) and in the control group (ADSC = 12.5, p = 0.0003) at 12 weeks.

Continued

Only one other study[30] compared two populations of cells (meniscal derived cells and MSCs). In this work, osteoarthritic defects came out in all knees, after the resection of the medial menisci (average OARSI grade 3.1). Nevertheless, meniscus punch defects were mostly repaired using either MSCs or meniscal cells. The new tissue, histologically, was meniscus-like with classic pericellular meniscal cavities, a significant amount of extracellular matrix and a low cell density. Immunostaining for collagen II was considerably positive. Characteristic radially orientated collagen fibers could be noticed in the architecture of the reconstructed meniscus. Gross joint assessment demonstrated donor site morbidity for meniscal cell treatment. The gene expression and production of collagen type II in human MSCs was significantly higher than in meniscal cells ($p<0.05$). The whole remaining cohort of studies did not compare different types of cells but, every population analyzed in the study independently, showed increased proliferation and differentiation. The results of these studies showed an increase in terms of proliferation and differentiation in all the cell groups (Table III). This trend is consistent with the whole group of clinical studies, which have shown good results in terms of imaging and clinical scores. None of them compared the population of cells. The most relevant study, in accordance with our quality analysis, was conducted by Vangsness et al[31] who in 2014 analyzed the function of MSCs injected on massive meniscal tears in 55 patients. They found significant improvement in scores at 3 months. MRI at 12 months showed significant increase in meniscal volume in MSC groups compared to control. The remaining cohort of studies had a low number of patients (two case controls, one case series and one case report for a total of 9 patients), but all of them showed improvement in clinical and imaging scores. The most relevant limitation of the present investigation is the low number of studies available on this topic and the low number of comparative studies. In particular, pre-clinical studies used different scores to evaluate proliferation and differentiation, which made a qualitative and quantitative comparison impossible. Moreover, clinical studies were mostly case reports, with low number of patients, lacking randomization and sufficient sample sizes. Finally, a lack of a subgroup analysis for the specific cell-delivery strategy could represent a high risk of bias.

Conclusions

This study underlies a high rate of differentiation, proliferation, radiological and clinical improvements in animals or patients treated with the application of stem cells for meniscal tears. At the current state of research, BMMSCs delivered through injection represent the most studied and promising therapy to invest on. Further research and higher quality studies should be carried out to compare application of different populations of cells and clinical benefits on humans.

Table IV. Pre-clinical study details. Pre-clinical study details.

Study	Procedures	N° of cells	Measures	Results
Centeno et al[40]	Percutaneous knee injection	45.6×10^6	VAS, Functional rating index, MRI	Increased meniscus volume on MRI. Decreased VAS Score from 3.33 to 0.13
Vangsness et al[31]	Percutaneous knee Injection	A:50×10^6 B: 150×10^6	MRI, VAS, Lysholm knee score	Significant improvement in scores at 3 months. MRI at 12 months: significant increase in meniscal volume in MSC groups compared to control
Pak et al[41]	Percutaneous knee Injection	—	VAS, Functional rating index, ROM, MRI	At 3 months MRI showed no evidence of meniscal tear, Symptoms improved and asymptomatic at 18 months
Onoi et al[42]	Percutaneous knee Injection	5.5×10^6	MRI KOOS Arthroscopy	Both patients reported better scores at 6 months follow up. 2nd look arthroscopy showed meniscal tear healing
Sekiya et al[43]	Arthroscopic transplantation of autologous synovial MSC suspension to sutured meniscal lesion	$32–70 \times 10^6$	Lysholm knee score, KOOS, NRS, 3D MRI	Significant improvement of Lysholm score by 1 year. Other scores significantly increased by 2 years. 3D MRI: Tears were indistinguishable

Funding

This project received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 814444 (MEFISTO).

Institutional Review Board Statement

Not applicable (systematic review of the literature).

Informed Consent Statement

Not applicable.

Data Availability Statement

All the data retrieved have been included in the manuscript.

Conflict of Interests

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualization, G.R. and P.G..; methodology, P.G.; software, G.C.; validation, B.D.M., P.C. and L.I..; formal analysis, P.C.; investigation, F.M.; resources, L.I.; data curation, L.I.; writing—original draft preparation, P.G.; writing—review and editing, G.R. and B.D.M; visualization A.C.; supervision, A.C.; project administration, P.G..; funding acquisition, B.D.M. All authors have read and agreed to the published version of the manuscript.

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