Cell-Based Meniscus Repair and Regeneration: At the Brink of Clinical Translation?

A Systematic Review of Preclinical Studies

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Background: Meniscus damage can be caused by trauma or degeneration and is therefore common among patients of all ages. Repair or regeneration of the menisci could be of great importance not only for pain relief or regaining function but also to prevent degenerative disease and osteoarthritis. Current treatment does not offer consistent long-term improvement. Although preclinical research focusing on augmentation of meniscal tear repair and regeneration after meniscectomy is encouraging, clinical translation remains difficult.

Purpose: To systematically evaluate the literature on in vivo meniscus regeneration and explore the optimal cell sources and conditions for clinical translation. We aimed at thorough evaluation of current evidence as well as clarifying the challenges for future preclinical and clinical studies.

Study Design: Systematic review.

Methods: A search was conducted using the electronic databases of MEDLINE, Embase, and the Cochrane Collaboration. Search terms included *meniscus*, *regeneration*, and *cell-based*.

Results: After screening 81 articles based on title and abstract, 51 articles on in vivo meniscus regeneration could be included; 2 additional articles were identified from the references. Repair and regeneration of the meniscus has been described by intra-articular injection of multipotent mesenchymal stromal (stem) cells from adipose tissue, bone marrow, synovium, or meniscus or the use of these cell types in combination with implantable or injectable scaffolds. The use of fibrochondrocytes, chondrocytes, and transfected myoblasts for meniscus repair and regeneration is limited to the combination with different scaffolds. The comparative in vitro and in vivo studies mentioned in this review indicate that the use of allogeneic cells is as successful as the use of autologous cells. In addition, the implantation or injection of cell-seeded scaffolds increased tissue regeneration and led to better structural organization compared with scaffold implantation or injection of a scaffold alone. None of the studies mentioned in this review compare the effectiveness of different (cell-seeded) scaffolds.

Conclusion: There is heterogeneity in animal models, cell types, and scaffolds used, and limited comparative studies are available. The comparative in vivo research that is currently available is insufficient to draw strong conclusions as to which cell type is the most promising. However, there is a vast amount of in vivo research on the use of different types of multipotent mesenchymal stromal (stem) cells in different experimental settings, and good results are reported in terms of tissue formation. None of these studies compare the effectiveness of different cell-scaffold combinations, making it hard to conclude which scaffold has the greatest potential.

Keywords: regenerative medicine; knee; meniscus; tissue scaffolds; stem cell therapy

The meniscus is essential for shock absorption, stability of the knee joint, and articular surface protection.[44] Meniscus damage is one of the most common injuries seen by orthopaedic surgeons, with an annual incidence of 66 to 70 per 100,000 people. Meniscal tears can be caused by trauma or degenerative disease. Traumatic meniscus injury is frequent among high school athletes, with an incidence of 5.1 per 100,000 in the United States.[44,46] Meniscus injury is an essential predictor of development of degenerative joint disease and is strongly correlated with the

incidence of subsequent osteoarthritis.[16,44] Thus, retaining, repairing, or even replacing the meniscus receives increasingly more attention. The proper prevention and treatment of meniscal damage addresses the large unmet medical need.

The ability of the torn meniscus to self-repair is limited.[36,40,44] Hypovascularity, hypocellularity, high density of the extracellular matrix, presence of inflammatory cytokines, and mechanical stress all contribute to low or absent self-repair, particularly in the avascular zone.[36,43,52,70] Current treatment strategies are primarily aimed at pain relief and improvement of joint function. Meniscectomy leads to loss of contact area, which eventually may lead to degenerative changes and osteoarthritis.[16] The incidence of osteoarthritis (both radiographic and symptomatic) has been shown to increase up to 7-fold after total meniscectomy in a 16-year follow-up cohort study.[17] The amount of resected tissue was a predictor of osteoarthritis.[55] Although partial meniscectomy showed to increase radiographic signs of osteoarthritis, it did not significantly increase symptoms at 8- to 16-year follow-up.[59] This is a drawback of this frequently used therapy, particularly in young, athletic patients. On the other hand, 2 recent randomized trials showed that physical therapy performs equally to partial meniscectomy in terms of pain reduction and functional improvement.[35,77] However, in the study by Katz et al,[35] 30% of the patients allocated to physical therapy still received a meniscectomy within 2 months, thus limiting the advantage of conservative treatment. Although the advancement of arthroscopic surgical procedures and increased attention to osteoarthritis have led to numerous new methods for meniscal repair, these are mainly indicated in young patients with a traumatic tear within, or close to, the vascular zone, and reported failure rates are high (20%-24%).[16,50] Different biomaterials, such as collagen and glycosaminoglycan scaffolds, allow for ingrowth and differentiation of cells, potentiating repair after meniscectomy. Although encouraging clinical results are achieved by scaffold implantation, a failure rate of up to 10% is reported, and data on long-term outcomes are lacking.[16,56]

The use of cells for regeneration or augmentation of repair holds great potential, as cells can adapt to the new environment and respond to signals sent by the damaged meniscus[27] or differentiate and actively contribute to the repair.[28] In this way, cell therapy may improve long-term outcomes of partial and total meniscectomy and allow for a broader indication of repair for both traumatic as well as degenerative meniscus tears.

Many studies describe the use of multipotent mesenchymal stromal (stem) cells (MSCs) because they are easy to isolate and expand and have the capacity to differentiate into the chondrogeneic lineage.[61] In the first human trial, Vangsness et al[72] studied intra-articular injection of MSCs in patients aged 18 to 60 years awaiting partial medial meniscectomy based on magnetic resonance imaging (MRI) findings. Included patients received intra-articular MSCs 7 to 10 days after partial meniscectomy. Safety data, meniscal volume, and clinical outcomes were evaluated during a 2-year follow-up period. Significant improvement with greater than 15% increased meniscal volume was reported in 24% of patients. Two case reports describe intra-articular injections of MSCs and report significant clinical improvement[54] and increased meniscal volume.[6] Fibrochondrocytes,[18,33,42,52] chondrocytes,[32,37,38,74] and myoblasts[23,82] have also been used for regeneration of the meniscus in animal models. While many preclinical studies have been performed, the abovementioned reports highlight the lack of evidence for cell-based meniscal repair augmentation and regeneration and warrant a thorough evaluation of the studies performed. Our systematic literature review was aimed at unraveling the most promising cell types or culture conditions described in vivo. Moreover, we will evaluate whether a cell carrier or scaffold could increase effectiveness of cell-based treatments, as it could support cellularity and tissue ingrowth while providing mechanical support to the meniscus.[43] The purpose of this review is to assess potential targets for optimization of cell-based meniscus repair augmentation and regeneration after partial and total meniscectomy. We included all in vivo models for meniscal tears in which repair was augmented by cell-based therapy, as well as total and partial meniscectomy models in which regeneration (formation of neomenisci) was targeted.

METHODS

A systematic review of literature aimed at cell-based systems for meniscal regeneration was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A search was conducted on May 18, 2016 in the electronic databases of MEDLINE, Embase, and the Cochrane Collaboration using the following search strategy: (Regenerative medicine'/exp OR regeneration OR regenerative OR regenerating OR repair OR repairing OR reparation OR replace OR replacement OR replacing OR augment OR augmentation OR augmenting OR restore OR restoring OR restoration OR "tissue engineering" OR regenerate) AND ('knee meniscus'/exp OR meniscus OR menisci) AND ('cells'/exp OR cell OR cells OR cellular). The articles were screened by title and abstract using the following inclusion criterion: articles describing a cell-based system for meniscus repair tested in vivo. Articles describing in vitro experiments were excluded, as were articles looking solely at femoral or tibial cartilage regeneration. Reviews, case reports, missing full texts, and articles in languages other than English were also excluded.

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RESULTS

The literature search yielded 525 articles in MEDLINE, 640 articles in Embase, and 2 articles in the Cochrane Collaboration. After removing 313 duplicates and screening titles and abstracts, the full text of 81 articles was screened, after which 51 articles could be included; 2 additional articles were identified from the references. Included articles and the different strategies for meniscus regeneration can be found in Table 1. Table 2 demonstrates the different outcomes measured in the in vivo experiments of all articles.

Cellular Meniscal Augmentation

Cell Types. MSCs isolated from different tissues are frequently used for regenerative purposes of the meniscus. MSCs are defined by their ability to form colonies and adhere to plastic, their expression of specific surface markers, and their trilineage potential.[13] MSCs from adipose tissue, bone marrow, synovium, and meniscus are used for repair augmentation of meniscal tears and regeneration after (partial) meniscectomy. The use of other cell types, including fibrochondrocytes,[18,33,42] chondrocytes,[38,74,75] and transfected myoblasts[82] is, in currently available literature, limited to the combination with different scaffolds. In the following paragraphs, we will focus on literature on in vivo augmentation of meniscal tear repair and regeneration after meniscectomy. We will elaborate on the effectiveness of MSCs of different sources and cell numbers needed for an optimal effect.

Synovial vs Bone Marrow MSCs. Synovial MSCs (SMSCs) can be extracted from the synovium during a simple arthroscopic procedure, but the synovium contains only a small amount of multipotent colony-forming cells.[8] Bone marrow MSCs (BMSCs) are frequently used in regenerative medicine and can be expanded from bone marrow in nonweightbearing areas.[75] In vitro, BMSCs form less colonies but show higher cell numbers per colony than SMSCs. SMSCs and BMSCs both have high chondrogeneic potential, as shown by the formation of pellets with a cartilaginous matrix. In a comparison of the effectiveness of intra-articular injection of SMSCs and BMSCs at passage 3 in a partial meniscectomy model in rats, both groups formed neomenisci. No notable differences were found between the BMSC injection group and the SMSC injection group at macroscopic evaluation. Histologically, both experimental groups showed healing and formation of type II collagen. The injected cells were still present at 12 weeks, as shown by LacZ tracking. Hierarchical clustering analysis showed that the gene expression profile of meniscal cells is closer to that of SMSCs than BMSCs.[29]

Bone Marrow vs Meniscal Multipotent MSCs. Meniscal MSCs (MMSCs) can be isolated from meniscal tissue resected at meniscectomy. In a study comparing BMSCs and MMSCs, both were demonstrated to have trilineage potential and to express markers indicative of stem cells (eg, Nanog, CD44, and CD90). BMSCs formed larger colonies and grew faster than MMSCs. However, when the MMSCs seeded in Matrigel were implanted subcutaneously in rats, MMSCs had a larger tendency toward the chondrogeneic lineage, whereas BMSCs had a greater tendency toward the osteogenic lineage.[12] This in vivo experiment, however, does not take into account the effect of load-bearing mechanics and the articular joint environment, limiting the relevancy of these findings.

Meniscus Cells, Adipocytes, Synovial Cells, and Chondrocytes. In the model used by Schwartz et al,[66] a bovine meniscus with bucket-handle tear was treated with adipocytes, meniscal cells, or synovial cells and placed on the dorsum of rats. The tears treated with meniscus cells had higher histological scores for integration than menisci treated with adipocytes or synovial cells. Marsano et al[41] demonstrated greater proliferation rates of chondrocytes than of meniscus, fat pad, and synovial cells in vitro. Moreover, chondrocytes placed on a hyaluronan scaffold formed meniscus-like tissue when placed subcutaneously in nude mice, whereas meniscus, fat pad, and synovial cells formed a tissue consisting mostly of fibroblastic cells with minimal extracellular matrix and no detectable glycosaminoglycans.

Cell Number. The majority of studies have used 15 to 50 million cells in large animal models (goats, dogs, pigs)[23,25,32,38,42,71] and around 0.5 to 5 million in small animals (rabbits, rats, mice).[18,26-29,51-53] Desando et al[11] stated that menisci of sheep injected with bone marrow concentrate after partial meniscectomy with greater cell numbers have higher regenerative ability, although this study was not sufficiently powered to find statistically significant evidence, and individual data were not shown. Agung et al[2] demonstrated that migration was only directed toward the anterior cruciate ligament (ACL) 4 weeks after injection of 1 million green fluorescent protein (GFP)–positive BMSCs in rats with ACL, meniscus, and femoral condyle injury. When 10 million cells were injected, migration was directed toward the ACL as well as the meniscus. Moreover, toluidine blue was observed around GFP-positive cells, indicating tissue regeneration by the BMSCs or embedding of the MSCs in existing extracellular matrix. These findings indicate that injection of a greater cell number is required to potentiate regeneration of the meniscus in cases with more complex knee injuries. However, converting these quantities to the same cell number per body weight in humans translates to the use of over 500 million cells to reach the same effect. Because this is not feasible in clinical practice, the effect of aggregates of 25,000 synovial MSCs was tested in a partial meniscectomy model. The implantation of 5 aggregates of 5000 cells and 50 aggregates of 500 cells increased chondrogenesis and led to longer cell survival. Moreover, more MSCs were found to be attached to the site of damage when compared with a cell suspension of 25,000 cells.[34] The authors thus hypothesized that the use of aggregates increased the effect of stem cells and decreased the quantity of cells needed. In another study performed by the same group,[39] the regenerative effect of placement of aggregates of SMSCs after partial meniscectomy was studied in aged primates. The aggregates led to formation of bigger neomenisci and higher histological scores (modified Pauli score). However, the control group (no treatment) also showed regeneration of the meniscus, indicating the

TABLE 1
Strategies for Meniscus Regeneration[a]

Study	Animal Model	Defect (Tear/Meniscectomy)	Strategy	Cell Source	Amount of Cells	Control	Duration of Follow-up
Desando et al[11]	Sheep	Unilateral medial meniscectomy	Bone marrow concentrate or BMSCs in hyaluronan	Iliac crest BMSCs; autologous	Bone marrow aspirate 39 $\times 10^6$ (6-53); 6×10^6 MSCs	Hyaluronan	12 wk
Kondo et al[39]	12- to 13-year-old primates	Partial meniscectomy (anterior half of medial meniscus)	Aggregates	SMSCs	$14 \times 0.25 \times 10^6$	No aggregates	16 wk
Qi et al[62]	Rabbits	Partial meniscectomy (anterior half of medial meniscus)	Targeted cell delivery; intra-articular injection of superparamagnetic iron oxide–labeled cells	ATMSCs	2×10^6	Unlabeled cells, saline	12 wk
Jülke et al[32]	Goats	Meniscal tear	Wrapping with porcine collagen membrane with or without expanded chondrocytes	Porcine articular chondrocytes	15×10^6	Inside-out suturing	6 mo
Ozeki et al[53]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Implantation of autologous Achilles tendon with allogeneic MSCs	Rat synovial MSCs	1×10^6	Achilles tendon graft	8 wk
Nakagawa et al[49]	Microminipigs	Meniscal tear	Suturing + injection of MSC suspension	Allogeneic SMSCs	20×10^6	Suture + suspension	12 wk
Ferris et al[20]	Horses	Meniscal tears	Surgery + injection of BMSCs	Autologous BMSCs	15 to 20×10^6	Surgery (previous data)	24 mo
Hatsushika et al[25]	Pigs	Partial meniscectomy (anterior half of medial meniscus)	3-time injection of allogeneic synovial MSCs with 2-week intervals	Allogeneic SMSCs	50×10^6	PBS	16 wk
Okuno et al[51]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of syngeneic MSCs, minor mismatch, major mismatch injection	SMSCs	5×10^6	PBS	4 wk
Shen et al[68]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of hMeSPCs 1 and 2 weeks after meniscectomy	Human meniscus stem/progenitor cells	6×10^6	PBS	12 wk
Zhu et al[82]	Dogs	Meniscal tear	Implantation of a PLA/PGA scaffold with myoblasts expressing hCDMP-2	Myoblasts		Suture only; PLA/PGA scaffold with myoblasts carrying an empty vector; PLA/PGA scaffold with addition of recombinant hCDMP-2	12 wk

(continued)

TABLE 1 (continued)

Study	Animal Model	Defect (Tear/ Meniscectomy)	Strategy	Cell Source	Amount of Cells	Control	Duration of Follow-up
Esposito et al[18]	Rabbits	Medial meniscectomy	Implantation of a PLDLA/PCL-T scaffold with chondrocytes	Allogeneic meniscus fibrochondrocytes	1×10^6/mL	Cell-free scaffold/ meniscectomy	24 wk
Hatsushika et al[24]	Rabbits	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of SMSCs	SMSCs	10×10^6	Meniscectomy	6 mo
Katagiri et al[34]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Implantation of aggregates	SMSCs	0.25×10^6	Injection of 5×10^6 cell suspension and 25,000 cell suspension	12 wk
Osawa et al[52]	Athymic rats	Meniscal tear	Intra-capsular injection of fetal meniscus cells	Fetal meniscus cells	0.5×10^6	PBS	4 wk
Shen et al[69]	Rabbits	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of MMSCs	Allogeneic MMSCs	6×10^6	PBS	12 wk
Moriguchi et al[47]	Pigs	4-mm cylindrical defect	Implantation of a tissue-engineered construct	Allogeneic SMSCs	0.2×10^6 cells + 3-wk culture	Empty defect	6 mo
Zellner et al[79]	Rabbits	4-mm longitudinal meniscal tear	Implantation of a hyaluronan/ collagen matrix with precultured BMSCs	Autologous BMSCs	0.1×10^6	Suture; empty matrix; matrix with BMSCs; matrix with PRP	12 wk
Horie et al[27]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of BMSCs	Rat or human BMSCs	2×10^6	PBS	8 wk
Gu et al[23]	Dogs	Partial meniscectomy (anterior horn of medial meniscus)	Implantation of PLGA scaffold with myoblasts	Autologous myoblasts	15×10^6 + 3-wk culture	Empty defect; empty scaffold	12 wk
Horie et al[28]	Rabbits	1.5-mm cylindrical defect	Injection of SMSCs	Allogeneic SMSCs	2×10^6	PBS	24 wk
Kon et al[38]	Sheep	Medial meniscectomy	Implantation HA PCL scaffold with expanded chondrocytes	Autologous chondrocytes	40×10^6 cells/ scaffold + 14-d spinner flask culture	Cell-free scaffold/ meniscectomy	12 mo
Hong et al[26]	Rabbits	Meniscal tear at anterior tibial attachment	Pull-out repair + implantation of scaffold and hBMSCs	Human BMSCs	2×10^6	Pull-out repair	8 wk
Ruiz-Ibán et al[63]	Rabbits	Meniscal tear	Suture + injection of ATMSCs in Matrigel	Allogeneic ATMSCs	0.1×10^6	Suture	12 wk

(continued)

TABLE 1 (continued)

Study	Animal Model	Defect (Tear/ Meniscectomy)	Strategy	Cell Source	Amount of Cells	Control	Duration of Follow-up
Zellner et al[80]	Rabbits	Meniscal punch	Matrices + BMSCs, PRP, BM aspirate	Autologous BMSCs	1.5×10^6	Cell-free hyaluronan collagen matrix	12 wk
Dutton et al[15]	Pigs	Meniscal tear	Suturing + injection of BMSCs in fibrin glue	Autologous BMSCs	1 to 2×10^6	Suture; suture + injection of fibrin glue	8 wk
Zhang et al[81]	Goats	Meniscal tear	Injection of BMSCs transfected with hIGF-1 in a calcium alginate gel	Autologous BMSCs	30×10^6 cells/ mL	Empty defect; injection of BMSCs in calcium alginate gel; injection of calcium alginate gel	16 wk
Horie et al[29]	Rats	Partial meniscectomy (anterior half of medial meniscus)	Intra-articular injection of SMSCs and BMSCs	Allogeneic SMSCs + BMSCs	5×10^6	PBS	12 wk
Kon et al[37]	Sheep	Medial meniscectomy	Implantation of HA PCL scaffold with expanded chondrocytes	Autologous chondrocytes (cartilage)	40×10^6 cells + 14-day spinner flask culture	Empty defect; empty scaffold	4 mo
Angele et al[4]	Rabbits	Partial meniscectomy (middle third of the medial meniscus)	Implantation of hyaluronan/gelatin scaffold with BMSCs	Autologous BMSCs	2.5×10^6 + 14-d culture	Empty defect; empty scaffold	12 wk
Weinand et al[74]	Pigs	Meniscal tear	Implantation of a vicryl mesh scaffold (polyglactin) with chondrocytes	Allogeneic vs autologous chondrocytes (from knee joint or ear)	2.1×10^6	Empty defect; suture; empty scaffold	12 wk
Martinek et al[42]	Sheep	Subtotal meniscectomy (medial meniscus resected leaving 2-mm peripheral meniscal ridge)	Implantation of CMI with fibrochondrocytes	Autologous fibrochondrocytes	10×10^6 + 3-wk culture	Empty defect; empty scaffold	3 mo
Weinand et al[75]	Pigs	Meniscal tear	Implantation of vicryl mesh scaffold (polyglactin) with chondrocytes	Articular, auricular, costal allogeneic chondrocytes	Scaffold in suspension of 5×10^6	Empty defect; suture; empty scaffold	12 wk
Kang et al[33]	Rabbits	Medial meniscectomy	Implantation of PLGA with fibrochondrocytes	Allogeneic meniscal fibrochondrocytes	2×10^6 on scaffold → 1-wk culture	—	36 wk
Abdel-Hamid et al[1]	Dogs	Meniscal tear	Injection of BM aspirate	Autologous centrifuged BM aspirate	2 mL of 4 mL aspirate	Empty defect; not centrifuged BM aspirate	12 wk

(continued)

TABLE 1 (continued)

Study	Animal Model	Defect (Tear/ Meniscectomy)	Strategy	Cell Source	Amount of Cells	Control	Duration of Follow-up
Peretti et al[58]	Pigs	Meniscal tear	Implantation of a devitalized allogeneic meniscal slice with chondrocytes	Autologous chondrocytes (articular cartilage)	Cultured in 2×10^6 chondrocytes	Empty defect; suture; empty scaffold	9 wk
Walsh et al[73]	Rabbits	Partial meniscectomy (anterior half of medial meniscus)	Implantation of type I collagen sponge with autologous BMSCs	BMSCs	0.5×10^6/mL, seeded 8-12 h	Periosteal autograft; empty scaffold	24 wk
Ishimura et al[30]	Rabbits	Meniscal tear	Injection of fibrin glue with BM aspirate	BMSCs	$\pm 1 \times 10^6$ cells (0.1 mL bone marrow aspirate)	Empty defect; empty scaffold	12 wk
Port et al[60]	Goats	Meniscal tear	Implantation of fibrin clot with BM aspirate	Autologous BM aspirate	Not specified	Empty defect; suture; empty scaffold	4 mo
Subcutaneous models							
Ding and Huang[12]	Rabbit cells in rat		Injection of cells in Matrigel	MMSCs vs BMSCs	0.3×10^6	—	3 wk
Schwartz et al[66]	Bovine menisci in athymic rats	Bucket-handle tear	MSCs on collagen scaffold; collagen gel; hyaluronic acid	Meniscus cells vs synovial cells vs adipocytes	1×10^6 in collagen scaffold, 0.1×10^6 in collagen gel, 2 to 3×10^6 cells alone, 100,000 in HA	Injection of MSCs without scaffold	9 wk
Gu et al[22]	Dog myoblasts in nude mice	NA	Implantation PLGA scaffold with myoblasts	Myoblasts induced with CDMP-2 and TGF-β1	15×10^6	PLGA scaffold with uninduced cells	12 wk
Ferris et al[19]	Horse menisci in nude mice	Meniscal sections	Fibrin glue and BMSCs	Allogeneic BMSCs	0.2×10^6	Fibrin glue	4 wk
Yoo et al[78]	Swine meniscal disks in nude mice	Meniscal disks	PLGA scaffold with chondrocytes	Articular chondrocytes (dynamic oscillating culturing)	$1.68 \times 10^6 \pm 0.25 \times 10^6$	Empty scaffold; static culturing	12 wk
Schoenfeld et al[65]	Human meniscal tissue in athymic mice	Meniscal cells	Implantation of PGA polymer scaffold with fibrochondrocytes	Human fibrochondrocytes	1×10^6 + 1-wk incubation	Empty scaffold	12 wk
Scotti et al[67]	Swine meniscal slices in nude mice	Meniscal slices	Articular fibrochondrocytes in fibrin glue + hydrogel	Swine meniscus fibrochondrocytes	2.4×10^6	Fibrin glue + hydrogel	4 wk
Marsano et al[41]	Human cells in nude mice	NA	Different cell types on nonwoven hyaluronan meshes	Human meniscus, fat pad and synovial membrane cells	4×10^6	NA	6 wk

(continued)

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Study	Animal Model	Defect (Tear/ Meniscectomy)	Strategy	Cell Source	Amount of Cells	Control	Duration of Follow-up
Peretti et al[57]	Lamb meniscus in nude mice model	Meniscal tear	Devitalized meniscal tissue with cultured chondrocytes, fibrin glue around the construct	Chondrocytes	Cultured in 12×10^6 chondrocytes	Empty defect; suture; empty scaffold	14 wk
OA models							
Ude et al[71]	Sheep	Medial meniscectomy (and excision of anterior cruciate ligament)	Intra-articular injection of chondrogenically induced cells	ATMSCs vs BMSC autologous	20×10^6	Medium	6 wk
Caminal et al[5]	Sheep	Meniscal tear (full-thickness articular cartilage defect)	Intra-articular injection of BMSCs	BMSCs	11×10^6 or 12×10^6	Medium	6-12 mo
Al Faqeh et al[3]	Sheep	Medial meniscectomy (and excision of anterior cruciate ligament)	Intra-articular injection of BMSCs	Autologous BMSCs	10×10^6	Basal medium	6 wk
Agung et al[2]	Rats	Meniscal tear (and anterior cruciate ligament tear, articular cartilage defect)	Intra-articular injection of BMSCs	Allogeneic GFP+ BMSCs	1×10^6 vs 10×10^6	Sham operation; saline	4 wk
Murphy et al[48]	Goats	Medial meniscectomy (and excision of anterior cruciate ligament)	Intra-articular injection of sodium hyaluronan with cells	Autologous BMSCs expressing eGFP	10×10^6	Sodium hyaluronan	26 wk

[a]—, no data; ATMSCs, adipose tissue–derived mesenchymal stem cells; BM, bone marrow; BMSCs, bone marrow–derived mesenchymal cells; CDMP-2, cartilage-derived morphogenetic protein–2; CMI, collagen meniscus implant; eGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; HA/PCL, hydroxyapatite/poly(ε-caprolactone); hCDMP-2, human cartilage–derived morphogenetic protein–2; hIGF-1, human insulin-like growth factor-1; hMeSPC, human meniscus stem/progenitor cell; MSC, mesenchymal stem cell; MMSCs, meniscal mesenchymal stem cells; NA, not applicable; OA, osteoarthritis; PBS, phosphate-buffered saline; PLA/PGA, polylactic acid/polyglycolic acid; PLDLA/PCL-T, poly(L-*co*-D,L-lactic acid)/poly(caprolactone-triol); PLGA, polylactic-*co*-glycolic acid; PRP, platelet-rich plasma; SMSC, synovial mesenchymal stem cell; TGF-β1, transforming growth factor–β1.

intrinsic regenerative capacity of menisci in cynomolgus macaques. Another approach is the use of tissue-engineered constructs (TECs) for augmentation of meniscus repair, which has been recently reported by Moriguchi et al.[47] In this study, 0.2 million SMSCs were cultured for 3 weeks in a high-density suspension culture with ascorbic acid to develop 3-dimensional constructs of SMSCs and extracellular matrix. Implantation of this TEC into a 4-mm cylindrical defect led to complete healing; the defect was filled with fibrocartilage after 6 months.

In conclusion, there is no consensus on the effect of injection of different cell numbers, as limited research is available and results are conflicting. Moreover, the amount of cells needed for repair augmentation might be different from the amount needed for regeneration after (partial) meniscectomy. The use of aggregates or TECs could increase the success of injecting MSCs in terms of regeneration. These techniques should be compared with injection of different cell numbers to prove their efficiency.

TABLE 2
Outcome Measures[a]

Study	Biomechanics	Macroscopic Evaluation	Histology	OARSI	Immunohistochemistry	Cell Tracking	MRI	EM
Desando et al[11]		×	×		×			
Kondo et al[39]		×	×				×	
Qi et al[62]		×	×		×	×	×	
Jülke et al[32]		×	×					
Ozeki et al[53]		×	×		×	×		
Nakagawa et al[49]	×	×	×			×	×	
Ferris et al[20]								
Hatsushika et al[25]		×	×	×	×		×	
Okuno et al[51]		×	×		×	×		
Shen et al[68]		×	×		×	×		×
Zhu et al[82]		×	×		×			
Esposito et al[18]		×	×					
Hatsushika et al[24]		×	×	×		×		
Katagiri et al[34]		×	×		×	×		
Osawa et al[52]			×		×	×		
Shen et al[69]	×	×	×		×	×		×
Moriguchi et al[47]		×	×					
Zellner et al[79]	×	×	×					
Horie et al[27]		×	×	×	×	×		
Gu et al[23]		×	×		×			
Horie et al[28]		×	×		×	×		
Kon et al[38]		×	×	×				
Hong et al[26]		×	×					
Ruiz-Ibán et al[63]		×	×		×	×		
Zellner et al[80]		×	×		×	×		×
Dutton et al[15]	×	×	×			×		
Zhang et al[81]		×	×		×		×	×
Horie et al[29]		×	×		×	×		×
Kon et al[37]		×	×		×	×		
Angele et al[4]		×	×		×			×
Weinand et al[74]		×	×			×		
Martinek et al[42]		×	×					
Weinand et al[75]	×	×	×					
Kang et al[33]	×	×	×		×			×
Abdel-Hamid et al[1]		×	×		×			
Peretti et al[58]		×	×		×			
Walsh et al[73]		×	×		×	×		
Ishimura et al[30]		×	×		×			
Port et al[60]	×	×	×		×			
Subcutaneous models								
Ding and Huang[12]			×		×			
Schwartz et al[66]		×	×					
Gu et al[22]		×	×		×			
Ferris et al[19]		×	×					
Yoo et al[78]		×	×		×	×		
Schoenfeld et al[65]		×	×		×			
Scotti et al[67]		×	×					×
Marsano et al[41]			×		×			
Peretti et al[57]		×	×		×			
OA models								
Ude et al[71]		×	×		×			
Caminal et al[5]		×	×				×	
Al Faqeh et al[3]		×	×					
Agung et al[2]			×		×	×		
Murphy et al[48]		×	×		×	×		

[a]EM, electron microscopy; MRI, magnetic resonance imaging; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

Cell Tracking/Mechanism of Action. In a variety of studies, DiI labeling or GFP was used to track cells and observe the fates of these cells. Horie et al[28] concluded that injected SMSCs were recruited toward the defect and that these SMSCs contribute actively to tissue repair by forming extracellular matrix. Other experiments, conducted by the

same group, indicated that injected BMSCs act as trophic mediators by increasing type II collagen expression by meniscal cells and hereby stimulating meniscus regeneration.[27] Hatsushika et al,[24] however, stated that intra-articularly injected SMSCs after partial meniscectomy induced formation of synovial tissue, which in turn differentiated into meniscal tissue. SMSCs did not differentiate into meniscal cells directly. In experiments by Desando et al,[11] intra-articular injection of bone marrow concentrate in hyaluronan was compared with injection of BMSCs in hyaluronan after partial meniscectomy. Here it was hypothesized that growth factors excreted by bone marrow concentrate have beneficial effects on meniscus regeneration. However, both treatments led to meniscus formation with good cell density and proteoglycan content, and both treatment types contributed to protection against progression of osteoarthritis, as measured by decreased fibrillations of cartilage surface, decreased proteoglycan loss, and decreased subchondral bone thickness compared with the control group.

Thus, MSCs may be recruited to the site of meniscal damage and may serve both as trophic mediators as well as differentiating meniscus cells, although strong evidence for these suggestions is lacking.

Allogeneic Versus Autologous

Use of autologous cells is safe since disease transmission is not possible, and immunological rejection is not of concern. Clinical translation is difficult, as harvested cells need to be expanded, leading to longer treatment delay and rendering single-step procedures impossible. Moreover, cell expansion in good manufacturing procedures (GMPs)–licensed laboratories generates high healthcare costs. The use of allografts or allogeneic cells may therefore be preferable. After partial meniscectomy, injection of autologous SMSCs and SMSCs matched based on major histocompatibility complex (MHC) class I genes gave similar modified Pauli scores for histology, whereas MHC class I mismatched SMSCs led to altered scores.[51] In a comparison between allogeneic and autologous chondrocytes on a vicryl scaffold in swine, no differences in healing of the meniscal tear were noted.[74] The mentioned studies could indicate that the use of allogeneic cells is as successful as the use of autologous cells. Moreover, the first trials for meniscus or cartilage defects show safe use of allogeneic cells, with no serious treatment-related adverse events.[10,72]

Cell Delivery

Ideally, scaffolds provide mechanical strength, deliver cells to the appropriate site, allow cellular organization, and provide stimuli for the growth and formation of meniscus tissue.[14,31,76] Implantable scaffolds provide stability and facilitate ingrowth of cells but they have to be surgically implanted and (pre)seeded with cells. Application of injectable scaffolds is less invasive. Moreover, these scaffolds are flexible enough to attach and adapt to irregular defects. However, fast degradation and poor biomechanical properties are a great drawback.[23] Tissue-extracted scaffolds provide natural structure and stability and facilitate ingrowth but have limited availability and need to be surgically implanted and (pre)seeded. Schwartz et al[66] reported that MSCs lead to superior repair when they are injected in suspension compared with injection in a collagen scaffold, collagen gel, or hyaluronic acid. However, no quantitative data were provided, and these results were obtained in a subcutaneous model, which, since biomechanics are not involved, underestimates the positive effect of scaffolds on joint stability and cell organization. Moreover, the number of cells seeded in the scaffolds was lower than the number of cells injected. To date, no other studies have compared the use of cells-scaffold combinations to the use of cells alone.

Implantable Scaffolds. The most commonly used scaffolds are polylactic acid (PLA), polyglycolic acid (PGA), and a combination of these (PLGA).[14] Hyaluronic acid is a natural polymeric hydrogel and could be added to these scaffolds. The use of cell-seeded polymer scaffolds to facilitate regeneration has yielded promising results. Angele et al[4] implanted a hyaluronan/gelatin scaffold seeded with BMSCs after removal of the middle third of the medial meniscus. This implantation led to formation of meniscus-like cartilage and integration into host tissue in rabbits. Similar results were reported by Zellner et al,[79] who found that hyaluronan/collagen scaffolds seeded with BMSC medium initiated fibrocartilage-like repair tissue in a 4-mm longitudinal meniscal tear, with good integration and biomechanical properties. Moreover, both studies demonstrate the superiority of the use of cell-seeded scaffolds compared with empty scaffolds in terms of macroscopic signs of healing and extracellular matrix organization.

Although MSCs are frequently used for tissue regeneration, the use of different cell sources has also been described. Gu et al[22] induced chondrogeneic differentiation of dog myoblasts by culturing them in a cartilage-derived morphogenetic protein–2 and transforming growth factor–β1–enriched medium. After lentiviral transfection of human cartilage–derived morphogenetic protein–2 into dog myoblasts, the myoblasts on a PLGA scaffold induced regeneration in meniscal tears in dog menisci.[82]

The use of fibrochondrocytes for meniscal repair seems obvious, but few studies have used this approach. This could be because the cells can only be found in the meniscus, and availability is limited. However, combinations of polymeric scaffolds with fibrochondrocytes have been demonstrated to offer potential benefit.[18,33,65]

In summary, the majority of these studies show increased tissue regeneration and better organization in the cells-scaffold combinations compared with empty scaffolds.[4,18,79] None of these studies compare the effectiveness of different scaffolds, making it hard to conclude which scaffold has the greatest potential.

Injectable Scaffolds. The first reports of injectable scaffolds as delivery vectors for stem cell application were in meniscal tears and used fibrin. Port et al[60] showed no effect of either the addition of a fibrin clot (formed by stirring autologous blood) or both a fibrin clot and BMSCs before suturing the meniscal defect. Ishimura et al[30] used fibrin glue (consisting of fibrinogen, aprotinin, factor XIII,

thrombin, and $CaCl_2$) in combination with BMSCs and reported complete filling of the tear with fibrocartilaginous tissue, whereas the meniscal tears treated with fibrin glue alone were filled with immature cartilaginous tissue. Dutton et al[15] examined the addition of BMSCs in fibrin glue after suturing an avascular tear and found macroscopic complete healing in 75% of the experimentally treated pig menisci, whereas none of the untreated menisci showed complete healing. Moreover, BMSC-treated menisci had a significantly greater Young's modulus than the non–cell-treated menisci, although achieving only 25% of the stiffness of the normal meniscus.

Murphy et al[48] induced osteoarthritis by excision of the medial meniscus and resection of the ACL in goats. After the injection of BMSCs in a sodium hyaluronan gel, macroscopic evaluation showed the formation of neomenisci. The meniscal tissue that was formed in the cell-treated group consisted of fibroblast-like cells surrounded by type I collagen and rounded cells surrounded by type II collagen, whereas the control group did not show this kind of tissue. Moreover, when compared with injection of hyaluronan gel, injection of BMSCs in hyaluronan gel reduced the progression of osteoarthritic degradation processes such as erosion of articular cartilage, osteophyte formation, and changes in subchondral bone. Ruiz-Ibán et al[63] induced macroscopically complete healing by injection of adipose tissue–derived MSCs (ATMSCs)-Matrigel combination after suturing a meniscal defect in a rabbit model. Moreover, meniscal fibrochondrocytes were present at the repaired area.

Thus, there have been different successful approaches in the use of various MSCs combined with injectable scaffolds.[15,48,63,81] However, more research is required comparing the use of different injectable scaffolds to implantable scaffolds seeded with or without cells such as MSCs.

Tissue-Extracted Scaffolds. Ozeki et al[53] used autologous Achilles tendon grafts in rat knees after resection of the anterior part of the medial meniscus. Grafts placed in a suspension of allogeneic SMSCs for 10 minutes prior to implantation had better histological scores compared with grafts alone, and the SMSCs could be tracked up to 8 weeks around the graft and native meniscus. Martinek et al[42] used a collagen meniscus implant that was fabricated from bovine Achilles tendon and type I collagen and seeded with autologous fibrochondrocytes. After implantation in the peripheral rim of a meniscus after subtotal meniscectomy, formation of meniscal tissue, enhanced vascularization, scaffold remodeling, and extracellular matrix production were reported. Peretti et al[57,58] implanted lamb meniscal slices seeded with chondrocytes in a subcutaneous nude mice model. After 14 weeks, in 7 of 8 meniscal slices, both sides of the incision were connected at gross inspection. Histological analysis showed integration of the scaffold in the meniscal samples and filling of the defect with chondrocytes. Jülke et al[32] used a porcine collagen membrane to wrap chondrocytes around a meniscal tear in goats and reported that the addition of chondrocytes led to more connecting tissue formation and tear margin contact.

Although the use of tissue-extracted scaffolds has potential benefits for meniscus repair augmentation and meniscus regeneration, the translation of these methods to clinical practice is complicated by the low availability of the biomaterials as well as the clinical applicability of cell-based treatments.

DISCUSSION

For repair and regeneration of meniscal damage, several cell-based approaches have been described in this review. The available literature indicates that cell-based therapies can stimulate formation of meniscus-like tissue with extracellular matrix, including types I and II collagen. Moreover, several studies show increased biomechanical properties of cell-treated menisci compared with non–cell-treated controls, although reported mechanical strengths are only 25% to 50% of the strength of native meniscus.[49,79] Several approaches are described, such as intra-articular injection of MSCs, implantation of TECs or aggregates of MSCs, injection of MSCs in an implantable scaffold, and implantation of chondrocytes and fibrochondrocytes on various scaffolds.

The majority of studies describe the use of MSCs and illustrate the value of these cells for tissue engineering. However, the question remains as to what limits clinical translation? One reason could be that few comparative studies have been conducted, making it hard to draw a conclusion as to which cell type yields the most promising results. Harvesting SMSCs can be achieved during an arthroscopic procedure. These cells have high proliferation rates, and the gene profile of SMSCs is similar to that of meniscal fibrochondrocytes. For both ATMSCs and SMSCs, the drawback of limited availability could be overcome by using allogeneic cells. BMSCs can be harvested from non-weightbearing articular surfaces.[75] The downside of using these cells is that they may have a greater tendency toward osteogenesis.[12] MMSCs improved healing in the meniscus,[68,69] and several studies have shown the safety and effectiveness of using allogeneic MMSCs.[25,51] Allogeneic MMSCs can be harvested from excised menisci or cadavers, making them a valuable tool in meniscus regeneration. Only 2 studies have compared the use of allogeneic cells to autologous cells, and the limited effect of allogeneic MSCs in the clinical trial by Vangsness et al[72] could stimulate studies comparing allogeneic and autologous MSCs.

In this review, the use of various scaffolds is discussed. Although the presented studies are heterogeneous in the use of scaffolds and cell types, nearly all studies indicate a superior role of cell-scaffold combinations compared with the use of scaffolds alone.[4,18,79] However, no research comparing different types of scaffolds (with cells) is available. Tissue-extracted scaffolds have proven their value in diverse in vivo studies, but their application is limited by their clinical (GMP) applicability. Injectable scaffolds can easily be injected and have the ability to mold to irregular defects. Cell-seeded implantable scaffolds also hold potential, since scaffolds seeded with fibrochondrocytes, chondrocytes, and even myoblasts have shown to increase regeneration of the meniscus. However, more research should be conducted comparing the use of cell-scaffold

combinations (either injectable, implantable, or tissue-extracted scaffolds) to the injection of cells alone. In addition, the need for preseeding scaffolds has its limitations because it requires cell expansion and the availability of a GMP-approved cell therapy facility.

This systematic review included meniscal tear models, meniscectomy models, and osteoarthritis models. The mechanisms of the cell-based therapy might be different in these models, as this includes augmentation of repair of traumatic or degenerative tears or formation of new tissue to fill the void after meniscectomy and help restore meniscal shape and function. However, regenerated tissue was often seen in repair augmentation of meniscal tears, as cell-based treatment led to organized meniscus tissue and not fibrotic scar tissue.[30,48,79]

Although results of the in vivo experiments discussed in this review look promising, only 1 clinical trial has been performed to date. The translation of in vivo studies to clinical practice has proven to be difficult. First, it is challenging to model meniscal pathology as seen in humans. In experimental settings, the treatment is given immediately or within 2 weeks after the meniscal injury. In clinical practice, treatment is usually provided months after trauma due to patient and/or doctor delay. In clinical practice, there remains an unmet clinical need for effective meniscal repair/regeneration. The regeneration rate is different in acute defects compared with chronic meniscal degeneration. Indeed, Ruiz-Ibán et al[63] demonstrated delay of treatment for 3 weeks leads to a decrease in the beneficial effect of ATSCs on meniscal damage. Chronic meniscus damage models provide a better reflection of the human pathology and could provide further insight in the potency of different regenerative medicine approaches.

Another limitation is the use of animal models that have innate regenerative capacity. In rat menisci, spontaneous regeneration occurs, which limits the translational value of these models.[27,29,68] This is particularly complicated in the use of biodegradable scaffolds, as these will be subjected to higher biomechanical strain. Therefore, as an animal model, rabbits may be preferred due to their limited regenerative capacity.[28] However, the rabbit meniscus has higher vascularization than the human meniscus and is relatively small, making it difficult to place the defect in the avascular zone.[7] Ghadially et al[21] demonstrated that menisci in rabbits, dogs, pigs, and sheep do not have innate regenerative capacity, which makes them suitable animal models. However, because the vascularity and cellularity of the meniscus decrease on maturation, the animals used have to be skeletally mature.[45,70] Moreover, the gait of these animals is different than that of humans, leading to a different pattern of mechanical load on the meniscus. A more suitable animal model was used by Kondo et al,[39] who studied meniscus regeneration in cynomolgus macaques. These primates are known to stand and walk mainly on their hind legs and are genetically closer to humans than nonprimates. The implantation of aggregates of 250,000 cells led to increased meniscal volume on MRI and better regenerated tissue quality both on macroscopic as well as on histological scoring. Moreover, the control group showed minimal increase in meniscal volume after 16 weeks. This is comparable to the human

situation, as Vangsness et al[72] did not observe an increase in meniscal volume greater than 15% in any patients in their control group. This indicates that these macaques, like humans, have no intrinsic regeneration.

Additionally, the currently available studies are limited by the lack of universal outcome measurements, making it difficult to compare different strategies. Moreover, few studies report mechanical testing of the regenerated menisci.[15,33,49,60,69,79] Although results look promising compared with suture alone or empty scaffolds, reported biomechanical strengths are still only 25% to 50% of the native meniscus. However, studies with a follow-up of 6 months or longer show ongoing proliferation, maturation, and organization of the regenerated tissue.[5,18,24,28,32,33,38,47] These findings suggest that biomechanics could improve up to 1 year after treatment, thus requiring longer follow-up periods to determine the effect.

Regulations on the use of minimally manipulated autologous cells are less strict, which allows for easier and faster clinical translation. However, the use of minimally manipulated autologous cells has not been described, as all included studies use precultured cells.

Although no research has been conducted to investigate the value of cocultures in cell-based meniscus regeneration in vivo, it could hold great potential because it exploits the benefit of MSCs as trophic mediators. Indeed, there have been promising results in articular cartilage repair in diverse in vivo models[9] as well as in a recent clinical trial (NCT02037204). For meniscal regeneration, cocultures of human BMSCs and fibrochondrocytes have also been tested in vitro, showing formation of neomenisci with enhanced extracellular matrix production compared with MSCs or fibrochondrocytes alone.[64] The use of cocultures could be of value in meniscus tissue engineering because it would allow a single-stage procedure. Autologous SMCs could be harvested arthroscopically and combined with allogeneic precultured fibrochondrocytes in an intra-articular injection. This way, the low MSC availability would no longer limit the clinical translation.

CONCLUSION

The studies included in this systematic review are heterogeneous in animal models, cell types, and scaffolds used, and there are limited comparative studies available. Although it is hard to conclude which strategy holds the greatest potential based on our findings, cell-based meniscus repair is promising. The use of cell-scaffold combinations was found to be superior to the use of empty scaffolds. Different cell-based therapies stimulated formation of meniscus-like tissue with organized extracellular matrix. Cell-based meniscus repair augmentation and regeneration is closer to translation than currently thought, and the first human clinical studies are now being performed. Minimally invasive and readily available strategies such as intra-articular injection of SMSCs or MMSCs hold potential as they can be used without need for preseeding of the scaffolds. Although strict regulations on the use allogeneic cells limit clinical translation, an advantage of

these cells is the possibility of preculturing them without the need for an extra harvesting and expansion procedure. The success of the first clinical trial by Vangsness et al[72] might be enhanced by injecting the cells in an injectable scaffold or onto an implantable scaffold, giving multiple injections, or combining MSCs with different cell types. Future research should aim at the efficiency of these regenerative procedures in large animals without innate regenerative capacity and in chronic damage models. Efficiency should be measured by a universal method of biomechanical testing, preferably with a minimum 6-month follow-up. In addition, early phase clinical trials are needed to bridge the gap between preclinical and clinical meniscus repair.

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