



Review

Mesenchymal stem cells in human meniscal regeneration: A systematic review

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ABSTRACT

Background: Stem cell regeneration is the holy grail of meniscal tissue repair. Currently, the best treatment is to preserve the original meniscus but if it fails, a partial meniscectomy is indicated to delay the onset of osteoarthritis.

Materials and methods: The authors present a systematic review to determine the up-to-date evidence underlying the use of mesenchymal stem cells for meniscal regeneration in humans. A search was conducted using the electronic databases of MEDLINE/Pubmed, Google scholar, and the Cochrane Collaboration. Search keywords included human, meniscus, stem cells and regeneration.

Results: After screening 10 non-duplicate studies, 5 were identified based on title and abstract. 4 were included in the analysis. There were marked differences in the method of stem cell harvest techniques. 3 studies administered stem cells through percutaneous injection into the knee and 1 study used a collagen scaffold. MRI analysis, functional scores and safety were assessed and the longest follow-up period was 2 years. The Visual Analogue Score (VAS) was most commonly used to assess function and patients generally showed an improvement. There were no reported adverse events.

Conclusion: Despite positive results from animal models, there is currently a lack of evidence in humans to conclude that stem cells can form durable neotissue similar to original human meniscus. There is a need for standardisation of protocol before further trials are considered. Initial outcomes from human studies are promising and mesenchymal stem cells may play an important role in meniscal repair in years to come.

1. Background

The holy grail of meniscal repair lies within the realms of meniscal regeneration. Most meniscal injuries are associated with a more active lifestyle and the damage of meniscal tissue renders young patients at a higher risk of undergoing meniscal surgery and hence osteoarthritis [1].

Established options for the management of a torn meniscus include partial or complete meniscectomy, meniscal allograft transplantation and synthetic meniscus transplantation. Other more conservative options include conduit treatment, abrasion therapy, platelet-rich-plasma therapy and more recently, meniscus tissue engineering [2]. Tissue engineering involves the use of cells with regenerative potential to augment the healing process following a meniscal injury. Cells that have been studied include articular chondrocytes, meniscal fibrochondrocytes, and mesenchymal stem cells (MSCs) [2]. Within orthopaedics, MSCs are mainly derived from bone marrow. However, other sources include synovial membrane [3], adipose tissue [4], meniscus-derived

MSCs [4] and extra-articular tissues such as dermis [5]. Adult MSCs are particularly attractive due to their potential for multilineage differentiation, immunomodulation and ability to migrate towards sites of injury [6]. One concern regarding MSCs is that the cartilage formed from MSCs has different mechanical properties to native meniscal tissue and inferior content in the extracellular matrix [6]. Further questions yet to be definitively answered include the optimal delivery method and scaffold choice [7].

A large volume of pre-clinical data exists, and stem cells in meniscal regeneration have been widely studied in animal models. Few studies, however, have assessed the in-vivo use of MSCs in human meniscal injuries. Limited understanding of the interplay between MSCs and stimulatory factors involved in meniscal regeneration is currently one of the factors contributing to regulatory burdens and their limited clinical use [8].

In this review, we explore the current studies which investigate the use of MSCs for meniscal regeneration in humans. Our objectives were:

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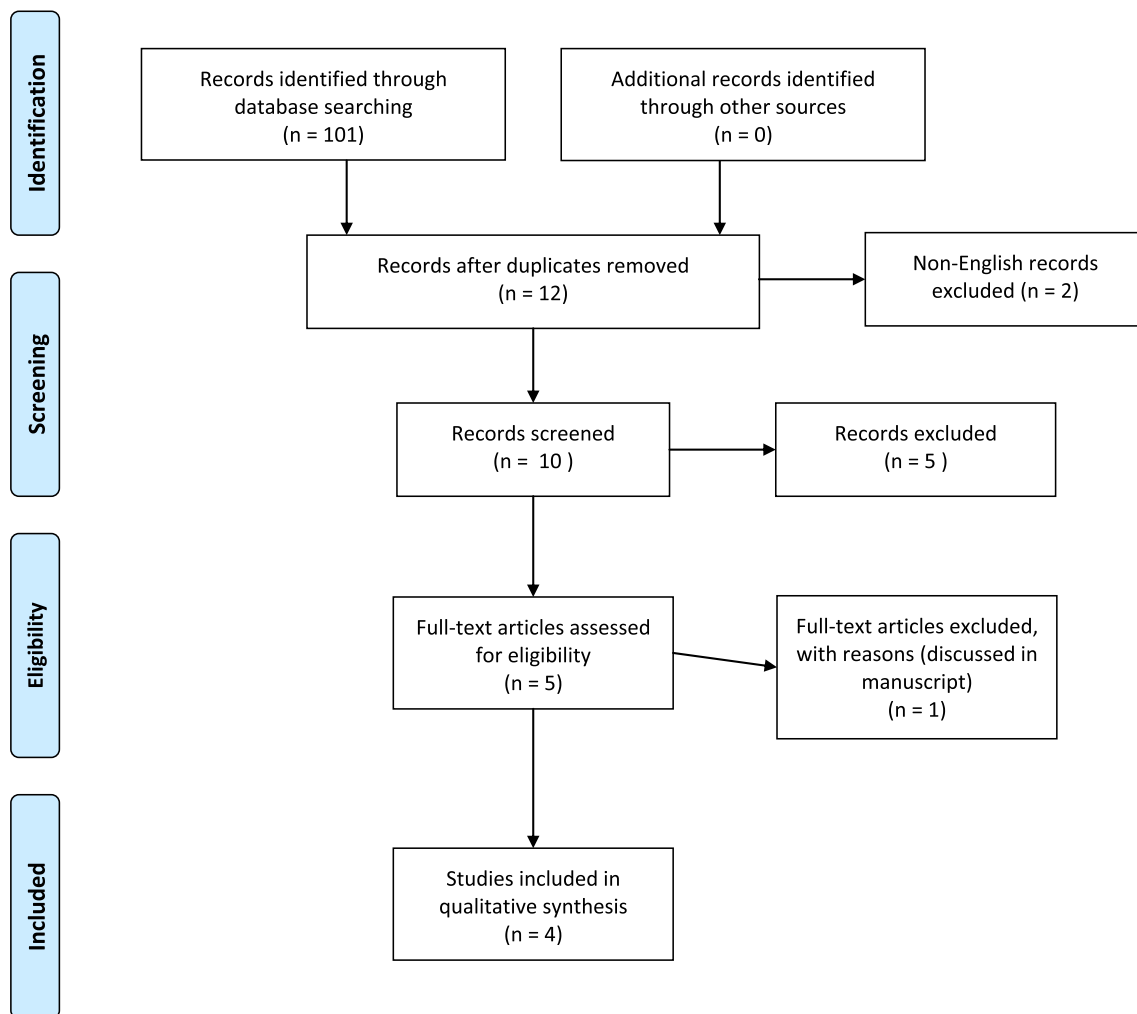


Fig. 1. PRISMA 2009 flow diagram.

to determine the efficacy of using human mesenchymal stem cells to repair damaged meniscus; to critically review all studies to date involving the application of mesenchymal stem cells into the human adult knee joint and to identify improvements for future human studies.

2. Materials and methods

A systematic review of literature for meniscal regeneration in the human model was performed and reported according to the PRISMA criteria (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [9]. Searches were carried out on 1st July 2017 of MEDLINE/Pubmed and Google Scholar using the following search string: Human AND (meniscal OR meniscus OR menisci OR knee) AND (tear OR injury) AND (stem cell OR mesenchymal OR MSC). The inclusion period was January 1st, 2000 to July 1st, 2017 (see Fig. 1).

Studies were then screened by title and abstract using the following inclusion criteria:

1. Mesenchymal stem cells used for meniscus repair, tested in human models only regardless of how cells were extracted or delivered.
2. Participants of any age, any nationality, male or female were included.
3. Participants must also have had MRI evidence of meniscal degeneration, meniscal tear or osteoarthritis, pre-stem cell application.

Studies involving the application of human stem cells in animal models, in-vitro experiments, reviews, articles in languages other than

English and articles with missing full texts were excluded. Each study was independently reviewed by the authors RP and EC, with relevant details recorded on a data extraction sheet.

3. Results

A total of 45 studies from MEDLINE, 55 studies from Google Scholar and 1 study from the Cochrane Collaboration were obtained from the literature search. After excluding 89 duplicated results and 2 non-English studies, 10 studies were analysed using their title and abstract, of which 5 studies were excluded as they involved the application of human stem cells in animal models. The full text of the remaining 5 articles were screened and 1 study was excluded as it was a study assessing the safety of stem cell administration. A total of 4 studies met the inclusion criteria in this review amounting to a total of 67 patients. 2 studies were case reports [10,11], 1 study was a case series [12] and 1 study was a double-blind randomized control trial [13]. The average patient age was 38. Gender distribution generally had more males than females. Table 1 outlines the methods of extraction and delivery of stem cells and Table 2 summarises the objectives and results of each study.

3.1. Method of obtaining stem cells

Vangness Jr et al.'s study used a preparation of ex-vivo cultured adult human mesenchymal stem cells derived from bone-marrow aspirates, obtained from unrelated donors who were not human leukocyte antigen (HLA)-matched to recipients. The donors were also between 18

Table 1
Methods of extraction and delivery of MSCs.

Year	Author	Type of study	Method of obtaining MSCs	Method of delivery of MSCs
2008	Centeno CJ et al.	Case Control	Bone marrow aspiration from the iliac crest.	Percutaneous injection into knee.
2014	Jaewoo Pak et al.	Case Control	Liposuction of the subcutaneous layer of the lower abdominal area.	Percutaneous injection into knee.
2014	Vangsness CT Jr et al.	Randomized controlled trial	Preparation of ex vivo cultured adult human mesenchymal stem cells derived from bone-marrow aspirates obtained from unrelated donors	Percutaneous injection into knee.
2017	Whitehouse MR et al.	Case series	Bone marrow aspiration from the iliac crest.	Arthroscopic application of MSC/ Collagen scaffold

and 30 years of age which differed from the average age of the recipient population. Jaewoo Pak et al. obtained stem cells from adipose tissue extracted by liposuction of the subcutaneous layer of the lower abdominal area. The stem cells were then separated from the lipoaspirates by a fat stem cell isolator after treatment with collagenase within the operating room and injected into the patient's affected knee. Centeno CJ et al. and Whitehouse MR et al.'s studies were the only studies to use bone marrow-derived stem cells from the patient. This was performed as a separate operation and bone marrow was obtained from the iliac crest. The stem cells were cultured to the third passage before being administered to the patient in Centeno CJ et al.'s study but Whitehouse MR et al.'s study showed no evidence of tumour forming potential at either passage 0 or 2.

3.2. Method of stem cell administration

Whitehouse MR et al.'s study was the only study to have used a collagen scaffold seeded with mesenchymal stem cells to repair the torn meniscus. All other studies used an intra-articular approach of percutaneously injecting stem cells into the affected knee joint.

3.3. MRI analysis

In Vangsness Jr et al.'s study, the formulated treatment for stem cell injection consisted of 50×10^6 cells (Group A), 150×10^6 cells (Group B) or the vehicle control (control group). At six months, an increase in meniscus volume of $> 15\%$ was observed in two patients, one each in groups A and B. At twelve months, four patients in Group A met the threshold of increase in meniscal volume. It is also mentioned that the control group compared with Group A and the overall group comparison (A + B) were significant at twelve months in terms of the proportion of patients meeting the criteria. At two years, three patients in Group A demonstrated an increase in meniscus volume of $> 15\%$ and

the combined group comparison (A + B) with controls remained significant. At no time point was the criterion achieved in any of the patients in the control group. Centeno CJ et al.'s study also showed an increase in meniscus volume at one and three months. Jaewoo Pak et al.'s study only had one MRI at three months which showed a healed meniscus but the patient refused to undergo further post-procedure MRIs due to symptom improvement and financial reasons. Whitehouse MR et al.'s study was the only study that did not measure meniscal thickness. However, for 3 out of the 5 subjects MRI did show that the menisci had not displaced and the initial abnormally high signal was diminishing with time after 12 months.

3.4. Functional scores

VAS and functional scores were reported in all 4 studies. In Centeno CJ et al.'s study, at three month follow-up modified VAS scores decreased from 3.33 to 0.13. Jaewoo Pak et al.'s study also had an assessment period of 3 months. Before the procedure, the patient had moderate pain (VAS score of 5) at rest and increased pain when walking (VAS walking index of 7), which all improved post-treatment. The study also reported an improvement of symptoms 18 months after treatment based on a telephone questionnaire. In Whitehouse MR et al.'s study the treatment failed in 2 patients who subsequently developed pain, swelling and locking in the knee at around 15 months leading to treatment with meniscectomy. The first case had a repeat tear at the site of repair and the second case had incomplete healing of the tear. Both patients started with a slightly lower baseline Tegner-Lysholm score and range of motion. In contrast, the remaining patients who were successfully treated showed improvements in all clinical scores over the first 12 months and these changes were maintained between 12 and 24 months. In Vangsness Jr et al.'s study, knee pain was assessed using a VAS tool and the Tegner-Lysholm knee score. Overall, VAS pain scores decreased significantly for patients post-surgery compared with baseline values

Table 2
Objectives and results.

Year	Author	Objectives	Results
2008	Centeno CJ et al.	Pre- and post-treatment subjective VAS pain scores, physical therapy assessments, and MRIs	At 24 weeks post-injection, the patient had statistically significant cartilage and meniscus growth on MRI, as well as increased range of motion and decreased modified VAS pain scores.
2014	Jaewoo Pak et al.	Pre- and post-treatment VAS walking index, Functional rating index, range of motion and MRI	Three months after the treatment, the patient's symptoms improved. Repeated MRI showed almost complete disappearance of the torn meniscus.
2014	Vangsness CT Jr et al.	Patients were randomized to one of three treatment groups: Group A- Injection of 50×10^6 allogeneic MSC Group B- 150×10^6 allogeneic MSC and the Control group- sodium hyaluronate vehicle control. Evaluate safety, meniscus regeneration, overall condition of the knee joint, and clinical outcomes at intervals through two years.	No ectopic tissue formation or clinically important safety issues were identified. There was significantly increased meniscal volume (defined a priori as a 15% threshold) determined by quantitative MRI in 24% of patients in Group A and 6% in Group B at twelve months post meniscectomy ($p = 0.022$). No patients in the control group met the 15% threshold for increased meniscal volume. Patients with osteoarthritic changes who received mesenchymal stem cells experienced a significant reduction in pain compared with those who received the control, on the basis of VAS assessments.
2017	Whitehouse MR et al.	Pre- and post-treatment IKDC and Tegner-Lysholm scores, range of motion and MRIs	3 out of the 5 patients had a successful outcome at 24 months showing improvements in all clinical scores over the first 12 months. Out of the 2 patients with failed implants, 1 had a tear at the implant site and the other had incomplete healing of the meniscus. These 2 patients showed no improvement in clinical scores between the 6–12 month period but had lower baseline Tegner-Lysholm scores compared to the other patients.

for all treatment groups. In patients with osteoarthritic changes at the time of surgery, improvement relative to the vehicle control was also observed for both groups A and B. Significant differences from the control were observed at two years for Group A and at one year and two years for Group B. Patients improved in their Tegner-Lysholm knee scale scores relative to the baseline at all time points.

3.5. Adverse effects and safety

No adverse events or clinically important safety issues were reported in any of the studies.

4. Discussion

It is difficult to make strong conclusions from the 3 case reports. Of note, Pak et al. and Whitehouse MR et al. investigated the effects of MSCs with relevance to meniscal repair, but Centeno et al.'s patient had evidence of osteoarthritis [14]. In the 2 case reports [6,9], a short follow up period of 3 months was used for MRI assessment, though Pak et al. conducted telephone follow up 18 months post-procedure. Nonetheless, excluding the studies by Vangsness et al. and Whitehouse MR et al., long term follow-up data to assess the efficacy and safety of MSCs in meniscal repair is lacking.

Post-procedure biopsies to histologically assess the nature of the regenerated tissue was also not performed in all three cases and may represent an obstacle which needs to be overcome in future studies. Visual analogue scores and functional rating scores were used as outcome endpoints in both studies. However, an internationally-accepted knee scoring system may lend greater validity and reliability to future studies. One such score is the WOMAC Osteoarthritis Index which is accepted as a reliable measure of knee function, pain and stiffness [15].

The type of stem cell used and the methods of extraction differed significantly between the four studies. Pak et al. and Centeno et al. injected platelet-rich plasma (PRP) and dexamethasone in addition to stem cells. Pak et al. also included hyaluronic acid in their preparation. The presence of additional components makes it difficult to attribute the clinical improvements solely to stem cell-derived tissue, although it should be noted that in the case reported by Pak et al. the patient had received previous injections of PRP and hyaluronic acid with no clinical improvement. A randomized controlled trial would be necessary to make any conclusions on whether injecting stem cells is superior to the application of a scaffold in a human model.

The characteristics of the meniscal tear in the case reported by Pak et al. were not discussed. However, Whitehouse MR et al.'s study did emphasise that the meniscal tears were in the avascular zone. In future studies this information will be important in understanding the potential of MSCs in the repair of the different types of meniscal tear. Thus it will be important that conclusions drawn from future studies take the characteristics of the tear into account.

All 3 case studies reinforce the short-term safety of using mesenchymal stem cells. However, as isolated case reports, they clearly provide limited information.

To date, Vangsness et al.'s study is the only level 1 study investigating the injection of allogenic MSCs in meniscal regeneration post-subtotal meniscectomy. The authors conclude that there was evidence of meniscal regeneration in the 2 groups treated with MSCs. However, closer analysis of the results showed that out of 35 patients only 5 had an increased meniscal volume > 15% at one year and this decreased to 3 patients over two years. Even though this was statistically significant, the clinical relevance is questionable. Furthermore, compared to some animal studies with a 2 year follow up [16], the decrease in meniscal regeneration could either suggest a short-term effect or variability in quantifying the meniscal volume on MRI. The type of stem cells used also weakens this study as they were pooled from unrelated donors and there was no mention of how they were obtained, which raises doubts on the pluripotency and quality of the cells.

Notably, long term pain improved in patients who had MSCs compared to controls [17]. The greatest improvement was observed in the group given the highest dose of MSCs, which could suggest a dose-dependent effect. However, the study does not quantify the extent of osteoarthritis across the groups. Additionally, patients within the control group had a BMI of more than 10 points lower than treatment groups, suggesting that the extent of osteoarthritis in the control group may be lower. There were also fewer patients with osteoarthritis in the control group (7 patients compared to 11 and 12 in the other two groups) which limits the comparison of outcomes.

It is undeniable that this level 1 study shows the safety of MSCs in treating meniscal injuries.

Limitations of the review include a small number of human studies, a lack of high quality studies and the included studies being incomparable due to variability in types of stem cells used and administration methods. These studies have shown that despite success in animal models [7], translational research in this area would need to consider more than just the efficacy of stem cells in meniscal repair.

Consensus must be reached on how MSCs are obtained and their cost-effectiveness. Jaewoo Pak et al. used liposuction to obtain stem cells in a separate operation before administration of stem cells. This would undoubtedly be a costly procedure both in terms of time and money. The ideal scenario would be harvesting and implanting stem cells in the least invasive way, making synovial membrane stem cells an attractive option. However, animal models have shown that synovium contains only a small amount of multipotent colony-forming cells [18] and bone marrow derived stem cells might be a more practical choice as they show larger cell numbers per colony [19]. This further raises the question on the best method of delivery, including scaffolds and direct knee injections. This is a widely debated topic and to date, no pre-clinical studies have compared the effectiveness of cell-scaffold combinations to the use of cells alone. Most importantly, the viability of newly formed neo-tissue needs to be assessed in longer term human studies. However, the current evidence suggests that stem cell therapy for meniscal repair in humans can be safely conducted, paving the way for more studies in the future.

5. Conclusion

Stem cells represent an exciting and attractive prospect in the treatment of meniscal injuries. Despite good results in animal studies, it remains in the early stages for humans. More studies need to be performed before a reliable assessment can be made. However, the limited number of human studies have suggested that stem cells do have potential to undergo meniscal regeneration in humans and there is reason to be optimistic for the future.

Ethical approval

Ethical approval not required.

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Author contribution

Ernest Chew, Ernest Chew: Study design, analysis, manuscript writing.

Rohan Prakash: Study design, analysis, manuscript writing.

Wasim Khan: Study design, manuscript editing.

Conflicts of interest

No conflicts of interest.

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Not applicable.

Guarantor

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References

- [1] M.P. Arnold, M.T. Hirschmann, P.C.M. Verdonk, See the whole picture: knee preserving therapy needs more than surface repair, *Knee Surg., Sport Traumatol. Arthrosc.* 20 (2) (2012) 195–196.
- [2] H. Yu, A.B. Adesida, N.M. Jomha, Meniscus repair using mesenchymal stem cells – a comprehensive review, *Stem Cell Res. Ther.* [Internet] 6 (1) (2015) 86. Available from: <http://stemcellres.com/content/6/1/86>.
- [3] Y. Sakaguchi, I. Sekiya, K. Yagishita, T. Muneta, Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source, *Arthritis Rheum.* 52 (8) (2005) 2521–2529.
- [4] Y. Segawa, T. Muneta, H. Makino, A. Nimura, T. Mochizuki, Y.J. Ju, et al., Mesenchymal stem cells derived from synovium, meniscus, anterior cruciate ligament, and articular chondrocytes share similar gene expression profiles, *J. Orthop. Res.* 27 (4) (2009) 435–441.
- [5] J. Sanchez-Adams, K. Athanasiou, Dermis isolated adult stem cells for cartilage tissue engineering, *Biomaterials* 33 (1) (2012) 109–119.
- [6] W. Fu, X. Xie, Q. Li, G. Chen, C. Zhang, X. Tang, et al., Isolation, characterization, and multipotent differentiation of mesenchymal stem cells derived from meniscal debris, *Stem Cells Int.* (2016) 2016.
- [7] J.V. Korpershoek, T.S. de Windt, M.H. Hagmeijer, L.A. Vonk, D.B.F. Saris, Cell-based meniscus repair and regeneration: at the brink of clinical Translation?: a systematic review of preclinical studies, Feb [cited 2017 Sep 16], *Orthop. J. Sport Med.* [Internet] 5 (2) (2017) 2325967117690131. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28321424>.
- [8] P. Angele, R. Kujat, M. Koch, J. Zellner, Role of mesenchymal stem cells in meniscal repair, *J. Exp. Orthop.* [Internet] 1 (1) (2014) 12. Available from: <http://www.jeosska.com/content/1/1/12>.
- [9] D. Moher, L. Shamseer, M. Clarke, D. Ghersi, A. Liberati, M. Petticrew, et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement, *Syst. Rev.* [Internet] 4 (1) (2015) 1. Available from: <http://systematicreviewsjournal.biomedcentral.com/articles/10.1186/2046-4053-4-1>.
- [10] C.J. Centeno, D. Busse, J. Kisiday, C. Keohan, M. Freeman, Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells, platelet lysate and dexamethasone, *Am. J. Case Rep.* 9 (2008) 246–251.
- [11] J. Pak, J.H. Lee, S.H. Lee, Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells, *Biomed. Res. Int.* [Internet] 2014 (2014) 436029. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3925627&tool=pmcentrez&rendertype=abstract>.
- [12] M.R. Whitehouse, N.R. Howells, M.C. Parry, E. Austin, W. Kafienah, K. Brady, et al., Repair of torn avascular meniscal cartilage using undifferentiated autologous mesenchymal stem cells: from in vitro optimization to a first-in-human study, *Stem Cells Transl. Med.* 6 (4) (2017) 1237–1248.
- [13] C.T. V Jr., J.F. II, J. Boyd, D.T. Dellaero, C.R. Mills, M. Leroux-williams, Adult Human Mesenchymal Stem Cells Delivered via, (2014), pp. 90–98.
- [14] C.J. Centeno, D. Busse, J. Kisiday, C. Keohan, M. Freeman, D. Karli, Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells, *Med. Hypotheses* [Internet] 71 (6) (2008) 900–908. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0306987708003654>.
- [15] J.M. Giesinger, D.F. Hamilton, B. Jost, H. Behrend, K. Giesinger, WOMAC, EQ-5D and knee society score thresholds for treatment success after total knee arthroplasty, *J. Arthroplasty* 30 (12) (2015) 2154–2158.
- [16] D.J. Ferris, D.D. Frisbie, J.D. Kisiday, C.W. McIlwraith, B.A. Hague, M.D. Major, et al., Clinical outcome after intra-articular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury, *Vet. Surg.* 43 (3) (2014) 255–265.
- [17] H.B. Ellis, Can a meniscus really regenerate so easily? A Level-I study says it can but not for everyone: commentary on an article, [cited 2016 Apr 13], in: C. Thomas Vangsness Jr MD, et al. (Ed.), *Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following.* *J. Bone Jt. Surg. Am.* [Internet], 96 2014 Jan 15 (2):e14. Available from: <http://jbj.org/content/96/2/e14.abstract>.
- [18] C. De Bari, F. Dell'Accio, P. Tylzanowski, F.P. Luyten, Multipotent mesenchymal stem cells from adult human synovial membrane, [cited 2017 Sep 16], *Arthritis Rheum.* [Internet] 44 (8) (2001 Aug) 1928–1942. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11508446>.
- [19] C. Weinand, G.M. Peretti, S.B. Adams, M.A. Randolph, E. Savvidis, T.J. Gill, Healing potential of transplanted allogeneic chondrocytes of three different sources in lesions of the avascular zone of the meniscus: a pilot study, [cited 2017 Sep 16], *Arch. Orthop. Trauma Surg.* [Internet] 126 (9) (2006 Oct 5) 599–605. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16411123>.