Minireview

Advances of human bone marrow-derived mesenchymal stem cells in the treatment of cartilage defects: A systematic review

Kaliappan Gopal, Haji Alizadeh Amirhamed and Tunku Kamarul

Department of Orthopedic Surgery, Tissue Engineering Group, National Orthopaedics Center for Excellence in Research and Learning (NOCERAL) & Clinical Investigative Centre, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia
Corresponding author: Tunku Kamarul. Email: tkzrea@um.edu.my

Abstract

Mesenchymal stem cell (MSC)-based therapies represent a new option for treating damaged cartilage. However, the outcomes following its clinical application have seldom been previously compared. The present paper presents the systematic review of current literatures on MSC-based therapy for cartilage repair in clinical applications. Ovid, Scopus, PubMed, ISI Web of Knowledge and Google Scholar online databases were searched using several keywords, which include ''cartilage'' and ''stem cells''. Only studies using bone marrow-derived MSC (BM-MSC) to treat cartilage defects clinically were included in this review. The clinical outcomes were compared, and the quality of the tissue repair was analysed where possible. Of the 996 articles, only six ($n = 6$) clinical studies have described the use of BM-MSC in clinical applications. Two studies were cohort observational trials, three were case series, and one was a case report. In the two comparative trials, BM-MSCs produced superior repair to cartilage treatment without cells and have comparable outcomes to autologous chondrocyte implantation. The case series and case–control studies have demonstrated that use of BM-MSCs resulted in better short- to long-term clinical outcomes with minimal complications. In addition, histological analyses in two studies have resulted in good repair tissue formation at the damaged site, composed mainly of hyaline-like cartilage. Although results of the respective studies are highly indicative that BM-MSC-based therapy is superior, due to the differences in methods and selection criteria used, it was not possible to make direct comparison between the studies. In conclusion, published studies do suggest that BM-MSCs could provide superior cartilage repair. However, due to limited number of reports, more robust studies might be required before a definitive conclusion can be drawn.

Keywords: Cell therapy, tissue repair, cartilage, autologous chondrocyte implantation, orthopaedic

***Experimental Biology and Medicine* 2014; 239: 663–669. DOI: 10.1177/1535370214530364**

Introduction

Arthroscopic studies conducted in recent years involving large number of patients have shown that the prevalence of cartilage defects can be high ranging anywhere between 11% and 63% in individuals presenting with some form of knee injury.[1] Cartilage, being an avascular, aneural and relatively hypocellular tissue; rarely undergoes complete repair following damage.[2] This poses a formidable challenge to the surgeons involved in the management and treatment of tissue damage. Various methods to repair articular cartilage have been previously described. However, it has been shown that long-term outcome has seldom been achieved, partly owing to the non-biological nature of the repair techniques currently employed.[3] In several studies, biological repair methods for cartilage defects involving the knee have been shown to produce superior tissue repair than those employing non-biological methods. In addition, it has also been shown that biological repair methods result in superior medium-term outcomes.[4] Thus, it is not surprising that biological repair techniques to treat damaged cartilage have recently become increasingly popular amongst orthopaedic surgeons. In many literatures, biological repair techniques are mainly referred to the use of one of the following: subchondral marrow stimulation, osteochondral graft transfer or autologus chondrocyte implantation (ACI).[3] It has been shown that ACI produced superior results to many other techniques, thus suggesting that cell therapy may be the best option to treating focal cartilage damage.[5] Nonetheless, despite its superior tissue repair outcome, the use of biological repair techniques do have certain limitations that restrict their potential from producing better long-term outcomes.[4,5] This limitation is explained by numerous reasons, one of which is in the use of chondrocyte itself, which has limited self-renewal capacity.[6] The reason for poor chondrocyte longevity appears to be due to its shortened telomeres, which following numerous cell

cycles, drives adult chondrocytes to undergo early senescence, obliterating long-term cartilage repair and remodelling.[6] To overcome this, the use of cells having improved self-renewal capacity may be preferable. Cells falling into this category include progenitor and other higher potency cells, such as mesenchymal stem cells (MSCs).[5]

In many studies, the use of bone marrow-derived MSCs (BM-MSCs) represents a new treatment modality for the repair of damaged cartilage. In many studies, superior cartilage repair outcomes becomes evident as observed from laboratory experiments as well as when used in clinical applications.[7] Being multi-potent and undifferentiated, BM-MSCs as with other types of MSCs, have the unique ability of being able to transform into cells of a particular lineage. These cells are able to reproduce the phenotypic expression similar to resident cells found in a specified tissue, i.e. cartilage cells.[8] MSCs are easily available and can be found in many tissues such as bone marrow, and therefore BM-MSCs offer a unique cell source that bypasses the necessity for multiple surgeries, a problem faced whilst employing ACI.[5,9] Despite the many promises of BM-MSCs to produce superior cartilage repair as demonstrated by the many published *in-vivo* studies, it appears that evidence relating to its use in clinical settings largely remains limited.[10] As a matter of fact, as far as the authors of this paper are aware, there have not been any reviews that have described presently published reports using BM-MSCs in treating cartilage damage. Here, we present a systematic review of publications reporting cartilage repair using BM-MSCs conducted primarily with the key objective of determining whether the use of BM-MSCs could lead to superior treatment outcomes clinically, as suggested from the results published by most laboratory investigations.

Materials and methods

Search strategy and study design

The online databases used in this study include Ovid, Scopus, PubMed, Google Scholar and ISI Web of Knowledge. The search terms ''Mesenchymal stem cell'' and ''cartilage'' were used, with restriction to articles published in English, since the year 2000. These results were searched for controlled trials using a highly sensitive and validated filter, and the articles were reviewed thoroughly and individually. The bibliographies of relevant original research articles were searched for further studies. Searches on the available papers were included and concluded by July 2013. The results of the selected articles reviewed here were critically scrutinized based on the treatment outputs such as clinical and patient follow-up outcomes (after MSC transplantation), tissue repair quality and study limitations.

Study selection

All studies that included the use of BM-MSCs for treating damaged cartilage in human were considered as eligible for review. Studies were included if they had been carried out in a clinical setting. Due to the very limited number of studies available, we extended the inclusion criteria to studies that were cohort trials, case series and case reports if the treatment group received MSCs and conducted in appropriate clinical settings. *In-vitro* and *in-vivo* investigations that used animal experiments were excluded from the analysis (Figure 1).

Data extraction

Data were extracted on (1) clinical outcomes as well as clinical evaluation scores, (2) quality of repair tissue (histological and arthroscopic outcomes where available) and (3) the length of patient follow-up after MSC transplantation with considerable number of patients. The outcome measures involving descriptive data were also taken into account where possible.

Results

Our online literature search produced 996 papers. After exclusion of duplicates, 343 were reviewed for suitability. In order to further determine if studies had met our eligibility criteria, articles were further stratified. Articles that were reviews ($n = 253$), non-clinical studies ($n = 69$) and animal study ($n = 15$) were excluded from the subsequent review process. The final evaluation resulted in six studies using BM-MSCs in treating patients for cartilage defects being included for review (Figure 1). Studies describing the use of BM-MSCs from sources other than bone marrow such as those which were peripheral derived were also excluded.[9] Of these, two were cohort trials, three were case series and the remaining, was a case report. Studies described the use of BM-MSCs to repair focal cartilage damage and in the cohort trials; one study compared the outcome of BM-MSC treatment with ACI whilst the other compared BM-MSC to using cell-free collagen sheets. All studies employed a similar technique in repairing cartilage defects, which involved the use of periosteal flap and the implantation of the BM-MSCs underneath the flap and into the defect sites. This is very similar to the technique employed in the original ACI described by others, although the authors have used BM-MSCs in lieu of autologous chondrocytes.[5] However, one of the cohort has also performed a high tibial osteotomy in addition to BM-MSC implantation. There were variations in the sites of operation, the comparison group, the outcome measures used as well as the length of follow-up (Table 2). Patient follow-up ranged from 6.3 weeks to 137 months after receiving treatment, involving 1–72 patients in each cohort, with the defect sizes that were treated ranging from 3 to 12 cm^2. There were many types of outcome measures used, which included patient assessment scores such as Lysholm or International Cartilage Repair Society (ICRS) scoring systems, and other more objective measures such as MRI and histological scores. A scrutiny of the study design and methodology employed made by at least two senior researchers in our institution, which had relevant research interest arrived to the conclusion that the studies selected were of relevance, better study design, and have employed valid and established outcome measures appropriate to the study they had undertaken. However, it is of interest to note that three ($n = 3$) of the six studies were from the Wakitani

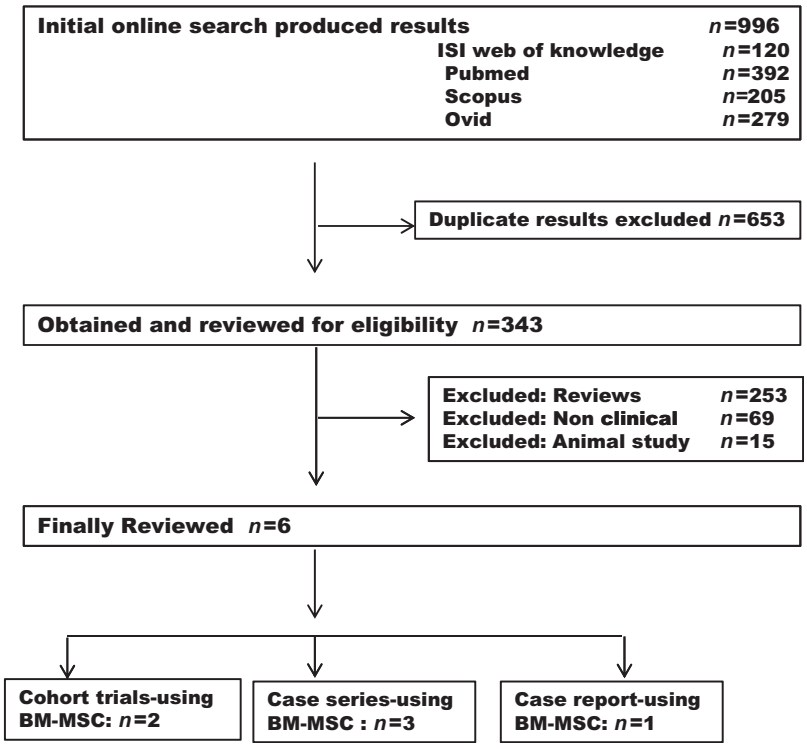

Figure 1 Flowchart of the literature collection through online search and selection of the reviewed articles

et al. group.[11–13] During our analysis it was not apparent to us if any or all of these studies were results being reported repeatedly and obtained from the same pool of patients. Because of this ambiguity, all three studies were included in the present paper. A summary of these results are summarized in Tables 1 and 2.

In this review, all the studies we reviewed appear to support the use of BM-MSCs to repair focal cartilage damage. However, the level of evidence varied with the study design employed by these studies as well as the number of participants enrolled. We found that the best evidence to demonstrate the efficacy of BM-MSC was of the reports from the two cohort trials. However, these were deemed to be of level-3 clinical evidence at best.[11,14] Overall, both reported cohort trials have concluded that BM-MSCs provide superior clinical outcomes as compared to; only collagen gel or ACI.[11,14] Between the studies by Nejadnik and Wakitani, we have found that the results published by the former was more convincing owing to its better study design, follow-up rates and, the rigid scrutiny and follow-up time-points employed by the authors.[11,14] The study by Wakitani *et al*.,[11] had several inconsistencies ranging from lack of patient numbers to incomparable treatment regimes. In their study, Wakitani *et al*.,[11] recruited what they described as patients with early osteoarthritis (OA) involving the medial compartment and not purely patients with chondral defects.[11] It is interesting to also note that whilst the authors have claimed that they recruited patients with OA, the overall impression was that patients were treated mainly for focal defect sites with few early OA changes at the edges of the condyles at best, as demonstrated from the pictures accompanying the published articles. Hence, this was the reason for us to include this article in the present review despite knowing that the article mentions of OA instead of focal cartilage defects. There were only 12 of the 24 patients treated with BM-MSCs whilst the others were treated with just cell-free collagen-sheets implanted underneath the periosteal flap, created using the same technique to that when ACI was performed. The difference to ACI was that a high tibial osteotomy was performed in all cases. At an average of 16 months of follow-up, although the BM-MSC treated group was deemed superior, there were no statistically significant differences observed between the two groups, which came to no surprise since the recruitment numbers were very small. On the other hand, the study by Nejadnik *et al*.[14] was better designed and had a control recruitment of 72 patients divided equally into two groups; one treated with BM-MSC and another with ACI. In their study, the outcome observed was based on a follow-up of 3-month interval culminating to a total follow-up of no $<$2 years. More importantly, the authors had clearly defined their recruitment targets, which only included patients with focal cartilage defects and not OA, which was very relevant to our review objective. Although they were not able to standardize the sizes and the sites of the treated defects, normalize the age groups and sexes, they demonstrated that the use of either BM-MSCs or chondrocytes results in comparable outcomes using similar outcome measurments. Despite having similar outcome at 2 years follow-up, the authors still support the use of BM-MSCs over chondrocytes citing that this is due to the ease of obtaining these cells as compared to chondrocytes for ACI. Furthermore, using BM-MSCs in treating cartilage defects is justified

Table 1 Characteristic of included studies addressing human bone marrow-derived mesenchymal stem cells based cell therapy for cartilage repair

Reference	Type of study	N (BM-MSC/control)	Controls treated with	Reported outcomes	Length of follow-up (month)	Average of defect size (cm^2, BMMSC/control)	First symptom of regeneration	Gender (m/f)	
1.	Wakitani *et al.*[11]	Cohort trials	24 (12/12)	Cell-free control	HSSK, arthroscopy, histology	16	4.42	After 6.3 weeks of transplantation	N/A
2.	Nejadnik *et al.*[14]	Cohort trials	72 (36/36)	ACI	ICRS package (Short Form-health survey), IKDC, Lysholm, Tegner	24	4.6/3.6	After 3 months of transplantation	38/34
3.	Wakitani *et al.*[13]	Case series	3	N/A	IKDC, arthroscopy, histology, MRI, X-ray	27	0.7–4.2	After 6 months of transplantation	2/1
4.	Wakitani *et al.*[12]	Case series	41	N/A	Subjective outcomes, X-ray	137	N/A		N/A
5.	Haleem *et al.*[15]	Case series	5	N/A	Lysholm, RHSSK, ICRS-arthroscopic score, X-ray, MRI	12	3–12	After 6 months of surgery	4/1
6.	Kuroda *et al.*[16]	Case report	1	N/A	ICRS-arthroscopic score, histology	12	6	After 7 months of surgery	1

ACI: autologous chondrocyte implantation; BM-MSCs: bone marrow-derived mesenchymal stem cells: HSSK: Hospital for Special Surgery knee-rating Scale; ICRS: International Cartilage Repair Society; IKDC: International Knee Documentation Committee; MRI: magnetic resonance imaging; RHSSK: Revised Hospital for Special Surgery Knee; N/A: not available.

since according to the authors, it avoids the use of additional surgical procedures needed in ACI.[14]

All remaining studies were of level 4 clinical evidence, involving patients with focal cartilage defects without any control or comparative group(s).[12,13,15,16] In the three case series investigations, two by Wakitani and others[12,13] and Haleem and colleagues,[15] it appears that all authors support the use of BM-MSCs since these cells provide good outcome for patients for up to a period of 137 months (please refer Table 2). In addition, they have also reported that the differences in the pre-operative assessment scores to that observed in the follow-up for >6 months, the use of BM-MSCs demonstrates significant improvements. However, it is worth noting that in the present review, Wakitani reports of two case-series; one published in 2007 and the other in 2010. It is unfortunate that we were unable to determine if the two are related since considering that similar surgical methods were employed in both studies. It may be the case that the later reported study is a mere follow-on report of the previous one and therefore in effect, there should have been only five studies worth mentioning in the present review instead of the six being assumed initially.[11–16] Lastly, the case report by Kuroda *et al*.[16] has made descriptive data using histological analysis of the BM-MSCs repaired site, which like all the reports reviewed herein, supports the use of these cells in treating focal cartilage defects (Table 2).

Discussion

The results published in the selected articles included in this review appear to unanimously agree that the use of BM-MSCs provides good repair outcomes, and in one study, the use of BM-MSCs has resulted in comparable outcomes to that of autologous chondrocytes. Although results of these studies appear robust and the findings appears to be valid, it is worth noting that to date there are limited number of published clinical data relating to this subject matter, i.e. only six studies (or publications) were found to be relevant.[11–16] In addition, the studies being reviewed had rather limited number of cohorts, with a highest number reported involving 72 patients divided into two treatment arms.[14] This is most unfortunate considering that the use of BM-MSCs has demonstrated such promising results. Had the number of patients recruited in these studies been larger, treatment using BM-MSCs could have been unequivocally advocated to become the future of cartilage repair. Those skeptical of the present report might also question the comparability of reported studies since the methodology employed are slightly varied. It is worth considering when conducting a review of such as this; it is not unexpected that when results from various research groups are compared across the board, certain differences in the methodology employed in the different studies may be apparent especially since there are no definite protocols that define how a study using BM-MSCs should be conducted. This should not be an issue since the emphasis of any review, such as that of the present article and that of many other studies for that matter, is to evaluate the outcome measures used in determining the efficacy of the

Table 2 Clinical and histological outcomes of included studies addressing BMSC-based cell therapy for cartilage repair

Type of cell treatment	Clinical outcome based on different scoring systems	Histological outcome	References
BM-MSC	No difference in clinical scores	Significant difference between BMS cell transferred group and cell-free group (ctrl). BMS group had hyaline-like cartilage whilst in the control group, repair was fibrocartilage like.	Wakitani *et al*.[11]
BM-MSC vs. ACI	No difference in clinical scores	Histology shows hyaline like cartilage tissue	Nejadnik *et al*.[14]
BM-MSC	Clinical symptoms improved over the follow-up period	Defect was repaired with the fibro-cartilaginous tissue	Wakitani *et al*.[13]
BM-MSC	Clinical symptoms was improved significantly, neither tumour nor infection was observed	No histology	Wakitani *et al*.[12]
BM-MSC	Clinical symptoms improved over the follow-up period	No histology	Haleem *et al*.[15]
BM-MSC	Clinical symptoms was improved significantly	The defect was filled with a hyaline-like type of cartilage tissue	Kuroda *et al*.[16]

BM-MSCs: bone marrow-derived mesenchymal stem cells; ACI: autologous chondrocyte implantation.

treatment used, i.e. the use of BM-MSCs in each of the study. The measures and reporting done must be valid and reflects the good outcomes of the repaired cartilage accurately. Our analysis demonstrates that there are no issues with regard to this since all reports that were reviewed had a study design that is sound and valid. The reports were also made with adequate length of time of follow-up in order for the final repair outcome to be observed, using appropriate outcome measuring tools for assessing cartilage repair. This was similar to that reported in most other studies.[4,5,11,12,14,17–19]

Despite this, it is to be expected that certain studies would be more preferred owing to the robust study design employed. A study which is of a clinical trial in nature involving comparative or control groups will provide more convincing results especially when this study involves large numbers of cohorts. Hence, in our opinion, the study by Nejadnik *et al*.[14] is by far the most convincing, since the study involves large population samples, a good sample variation and that the study had made a good comparison between BM-MSC and ACI in a side-to-side comparative analysis. In their study, it is demonstrated that the outcome of BM-MSCs-treated patients are not significantly different from patients treated with ACI but, it is said that treating with BM-MSCs will avoid the use of additional surgical procedures needed for ACI. More importantly, the study has demonstrated that the use of BM-MSCs could be used in older patients with little consequence on the final outcome, for which this remains an issue whilst using ACI. As far as we are aware, to date this issue has not yet been resolved. It is well recognized that ACI is less successful when used in patients >45 years of age.[19] The reason, as explained earlier, may be related to shortened telomeres, which are observed in most adult cells and even many types of unipotent cells.[6] In many studies, it has been suggested that using methods such as subchondral drilling and microfracture employs the same paradigm to that of implanting MSCs into the defective sites.[20,21] Their argument is that when a communicating channel is created between the defect site to the bone marrow canal, stem cells (in particular MSCs) migrate through these channels to occupy the lesions and repair the damaged sites in a way similar to that when implanting MSCs.[20,21] Whilst this argument appears to be sound in a single glance, there are many issues relating to this notion that delineates BM-MSC implantation from their migration from bone marrow. Firstly, the technique of implanting BM-MSCs employs cellular expansion and that high concentrations of specific cells homogenous in nature are to be used to treat damaged cartilage. In contrast, MSC migration from bone marrow as the result of microfracture of subchondral drillings is a speculated event, and the migrating cells are neither homogenous comprising of only MSCs nor are of high concentration.[20,22] Secondly, from the reports of long-term repair outcome observational studies it is apparent that techniques using microfracture do no result in medium-term hyaline-like cartilage healing, which unlike that those resulted from using BM-MSCs or ACI maintains a better quality of hyaline cartilage tissue repair.[4,23] Had both microfracture and implanting BM-MSCs work by the same principle, the final clinical outcomes should have been similar. However, the arguments put forth here are merely based on logical deduction and that in order to make conclusive arguments to this, a direct study employing randomized control trial comparing the two techniques would be necessary. From our literature search, we however found no studies as such, and the best that could be deduced is the use of previously published systematic reviews or meta-analyses. There were several such studies that were found to be relevant.[4,23] These studies compare ACI to other techniques such as microfracture, subchondral drilling and chondroplasty in a cohort control trial. The study by Nejadnik *et al*.[14] is the only one that has compared BM-MSCs to ACI whilst the others have reported that ACI was superior to all others. Hence, based on these findings we deduced that since BM-MSCs were comparable to ACI and that ACI is superior to

others, it may be the case when BM-MSCs and ACI may well be superior to other techniques. Nevertheless, due to the varied methods employed in each study, we were not able to perform a meta-analysis of these studies, which could have demonstrated more convincing results and conclusions.[14]

Despite the robust findings of the present report, several limitations were reviewed warranting a rationale whilst determining the effectiveness of BM-MSCs in treating damaged cartilage. A major drawback in all six reports is the relatively small sample size, even the one reported by Nejadnik *et al*.[14] There have been a number of confounding factors, which were not taken into consideration by Nejadnik *et al*.[14] that might warrant the removal of these cases from the cohort, for instance, excluding patients with the habit of smoking or stratifying their cohort according to body mass index, comorbidities or even the involvement of other potentially related underlying knee disorders. This would have resulted in fewer patients being used in the final analyses than that reported by the authors. In that paper, the authors themselves have stated that not all the patients received only BM-MSCs or chondrocytes to treat their medical condition. A number of patients were reported to have received concomitant procedures, which included patellar realignment, high tibial osteotomy, partial menisectomy and anterior cruciate ligament reconstruction.[14] This suggests that the treatments were not standardized entirely for the study population, or that the severity of damage could have been more severe in many patients. These factors could have had an influence on the final outcome. Another issue we observed in this review is when we attempted to make a cross tabulation of the outcomes reported by the different studies to determine if there was a way to compare the results objectively across the board. With varied outcome measures used and the presence of several methodological limitations in terms of total amount of cell transplanted, the differences in the defect size treated and their locations, and the differences in the methods employed in tissue repair scoring system; it was not possible to make any conclusive comparison. This was unfortunate since the results would have reflected more robust data that would be convincing for the readers, especially when the data are presented in the form of a meta-analysis. Lastly, since there were no studies comparing BM-MSCs with other biological repair methods that do not use cells such as subchondral drilling, it is less likely to convince skeptics about the use of the BM-MSCs since technique that does not involve cell transplantation would be easier and more readily available for the treating orthopaedic surgeons to provide. To counter this problem, we have attempted to make a case for supporting the use of BM-MSCs by quoting several previous reviews that have made comparison between ACI and other techniques.[4,23] With several reviews already showing that ACI is superior to abrasionplasty, microfracture and mosaicplasty; it is only sensible to assume that a study that shows BM-MSCs having an equal outcome to ACI suggests that BM-MSCs implantation would provide superior outcome than that of the earlier mentioned techniques. Unfortunately, the paper by Nejadnik *et al*. is the only paper we are aware to have published the outcome between BM-MSCs implantation and ACI, and therefore more publications to support this finding is much needed in order to convince a larger pool of researchers.

Nevertheless, despite having several shortcomings, the reports of BM-MSCs being used to treat damage cartilage appears to the hopeful and that the findings from these studies are highly indicative that the use of BM-MSCs is a good choice for treating damaged cartilage. Although further published results using more rigorous study designs and comparable outcome measures may become necessary to convince many skeptics, at present it is safe to say that treating cartilage using BM-MSCs is an acceptable alternative to the better known ACI. Considering the fact that using BM-MSCs has many advantages over ACI, which includes reduced donor site morbidity and the risk of additional surgery, it is worthwhile for patients to consider using BM-MSCs as a viable option. However, considering that only two cohort studies using BM-MSC have ever been reported since 2000 and, more studies may be required to make sure that BM-MSC therapy is safe for patients.

Author contributions: KG and HAA, literature search and generated the initial manuscript draft and the tables. KG and TK, participated in the design, interpretation and writing of the review. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors are grateful to the University of Malaya Research Grant (UMRG256/10HTM) and HIR-MoE Grant (Reference number – UM.C/625/1/HIR/ MOHE/MED/04, account number – E000003-20001) for supporting this study.

REFERENCES

1. Bos PK, Melle ML, Osch G-JVM. Articular cartilage repair and the evolving role of regenerative medicine. *Open Access Surg* 2010;**3**:109–22
2. Buckwalter JA, Mankin H, Grodzinsky AJ. Articular cartilage and osteoarthritis. *Instr Course Lect* 2005;**54**:465–80
3. Wakitani S, Kawaguchi A, Tokuhara Y, Takaoka K. Present status of and future direction for articular cartilage repair. *J Bone Miner Metab* 2008;**26**:115–222
4. Naveen S, Robson N, Kamarul T. Comparative analysis of autologous chondrocyte implantation and other treatment modalities: a systematic review. *Eur J Orthopaed Surg Traumatol* 2012;**22**:89–96
5. Lee EH, Hui JH. The potential of stem cells in orthopaedic surgery. *J Bone Joint Surg Br* 2006;**88**:841–51
6. Martin JA, Klingelhutz AJ, Moussavi-Harami F, Buckwalter JA. Effects of oxidative damage and telomerase activity on human articular cartilage chondrocyte senescence. *J Gerontol A Biol Sci Med Sci* 2004;**59**:324–37
7. Froilan GM, Weis JA, Longobardi L, Spagnoli A. Role of mesenchymal stem cells in regenerative medicine: application to bone and cartilage repair. *Expert Opin Biol Ther* 2008;**8**:255–68
8. Sacchetti B, Funari A, Michienzi S, Cesare SD, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 2007;**131**:324–36
9. Chong PP, Selvaratnam L, Abbas AA, Kamarul T. Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. *J Orthop Res* 2012;**30**:634–42

10. Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. *Arthrit Res Ther* 2008;**10**:223–35
11. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;**10**:199–206
12. Wakitani S, Okabe T, Horibe S, Mitsuoka T, Saito M, Koyama T, Nawata M, Tensho K, Kato H, Uematsu K, Kuroda R, Kurosaka M, Yoshiya S, Hattori K, Ohgushi H. Safety of autologous bone marrow-derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months. *J Tissue Eng Regenerative Med* 2011;**5**:146–50
13. Wakitani S, Nawata M, Kawaguchi A, Okabe T, Takaoka K, Tsuchiya T, Nakaoka R, Masuda H, Miyazaki K. Serum keratan sulfate is a promising marker of early articular cartilage breakdown. *Rheumatology* 2007;**46**:1652–1656
14. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med* 2010;**38**:1110–6
15. Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, El Shewy MT, Azzam A, Abdel Aziz MT. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. *Cartilage* 2010;**1**:253–61
16. Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, Ohgushi H, Wakitani S, Kurosaka M. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage* 2007;**15**:226–31
17. Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. *Cell Tissue Res* 2008;**333**:207–15
18. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, Goldberg VM. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;**76**:579–92
19. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;**331**:889–95
20. Nam HY, Karunanithi P, Loo WCP, Naveen SV, Chen HC, Hussin P, Chan L, Kamarul T. The effects of staged intra-articular injection of cultured autologous mesenchymal stromal cells on the repair of damaged cartilage: a pilot study in caprine model. *Arthritis Res Ther* 2013;**15**:R129
21. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. *Clin Orthop Relat Res* 2001;**391**:362–9
22. Boo L, Selvaratnam L, Tai CC, Ahmad TS, Kamarul T. Expansion and preservation of multipotentiality of rabbit bone-marrow derived mesenchymal stem cells in dextran-based microcarrier spin culture. *J Mater Sci Mater Med* 2011;**22**:1343–56
23. Goyal D, Goyal A, Keyhani S, Lee EH, Hui JH. Evidence-based status of second-and third-generation autologous chondrocyte implantation over first generation: a systematic review of level I and II studies. *Arthroscopy* 2013;**29**:1872–8

(Received August 19, 2013, Accepted February 3, 2014)