Sains Malaysiana 50(6)(2021): 1727-1744
http://doi.org/10.17576/jsm-2021-5006-19

Assessing the Potential of Bone Marrow Concentrate for Cartilage Repair and Regeneration in Animal Models: A Systemic Review

(Menilai Potensi Kepekatan Sumsum Tulang untuk Pembaikan dan Pembaharuan Tulang Rawan dalam Model Haiwan: Suatu Kajian Sistemik)

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ABSTRACT

Bone marrow concentrate (BMC) has been emerging as a promising regenerative source to accelerate cartilage regeneration in cartilage injuries and osteoarthritis. Though the number of stem cells in BMC is limited, BMC is rich in growth factors that promote stem cell differentiation and tissue regeneration. Despite of multiple reports available on the use of BMC for cartilage repair in humans and its use in clinical settings, only limited number of pre-clinical proof of concept studies have been reported in animal models. Hence, a systematic review focusing on the potential of BMC for the treatment of cartilage defect in animal models has been conducted. The systematic search of literature using three popular databases, ISI Web of Knowledge, PubMed and Scopus, were conducted without year restriction. Fifteen (n = 15) studies were found appropriate and included in this review. All of the included studies were of different animal models with cartilage defect. 13 out of 15 studies reported that the usage of BMC gave the best outcome compared to other treatment methods. Most of the findings provided good scoring on the tissue repair and the histological outcome. However, most of the BMC group outcomes did not give a significant difference when compared with other interventions such as the addition of platelet rich plasma, erythropoietin, hyaluronic acid, transforming growth factor, autologous tissue implant, genetic modification or scaffoldings. In conclusion, the published studies do suggest that BMC could provide a better cartilage repair. However, more preclinical studies are required to provide definitive conclusions.

Keywords: Animal study; bone marrow aspirate; bone marrow concentrate; cartilage; systematic review

ABSTRAK

Konsentrasi sumsum tulang (BMC) telah muncul sebagai sumber penjanaan semula yang berpotensi untuk mempercepatkan pertumbuhan tulang rawan dalam kecederaan rawan dan osteoartritis. Walaupun jumlah sel stem di BMC adalah terhad, BMC kaya dengan faktor pertumbuhan yang membantu dalam proses pembezaan sel stem dan penjanaan semula tisu. Walaupun terdapat banyak laporan mengenai penggunaan BMC untuk pembaikan tulang rawan pada manusia dan penggunaannya dalam tetapan klinikal, hanya sebilangan kecil bukti kajian konsep pra-klinikal yang terhad telah dilaporkan dalam model haiwan. Oleh itu, semakan sistematik yang menumpukan kepada potensi BMC untuk rawatan kecacatan tulang rawan dalam model haiwan telah dijalankan. Pencarian sastera sistematik menggunakan tiga pangkalan data popular, ISI Web of Knowledge, PubMed dan Scopus, telah dijalankan tanpa pembatasan tahun. Sebanyak lima belas (n = 15) kajian didapati bersesuaian dan disertakan dalam kajian ini. Semua kajian yang terpilih termasuk model haiwan yang berbeza dengan kecacatan tulang rawan. 13 daripada 15 kajian melaporkan bahawa penggunaan BMC memberi hasil yang terbaik berbanding kaedah rawatan lain. Kebanyakan penemuan memberikan skor yang baik ke atas pembaikan tisu dan hasil histologi. Walau bagaimanapun, kebanyakan hasil kumpulan BMC tidak memberikan perbezaan yang signifikan jika dibandingkan dengan campur tangan lain seperti penambahan platelet kaya plasma, eritropoietin, asid hialuronik, mengubah faktor pertumbuhan, implan tisu autologous, pengubahsuaian genetik atau perancah. Kesimpulannya, kajian yang diterbitkan menunjukkan bahawa BMC dapat memberikan pembaikan rawan yang lebih baik. Walau bagaimanapun, lebih banyak kajian pra-klinikal diperlukan untuk memberikan kesimpulan yang pasti.

Kata kunci: Haiwan; konsentrasi sumsum aspirat; konsentrasi sumsum tulang; rawan; semakan sistematik

Introduction

Bone marrow concentrate (BMC) also reported as bone marrow aspirate concentrate (BMAC) and concentrated bone marrow aspirate (CBMA) has been recently gaining its importance in the treatment of cartilage related diseases as it contains undifferentiated stem cells and growth factors which could be directly delivered *via* intra-articular injection at injury site. The usage of a minimally processed bone marrow concentrate (BMC) skips the lengthy and costly procedure of culturing methods, which then can be directly prepared and ready for use in the operating theatre, is fascinating. Number of studies has been reported on the use of bone marrow concentrate for cartilage repair. The potential of bone marrow is limitless due to its ability to provide stem cells that are capable of chondrogenesis and as source of growth factors that stimulates cartilage repair. It is believed to be having if not superior, equal to other cartilage repairing procedures (Huh et al. 2016; Madry et al. 2017). Another advantage of using the BMC is that chondro-progenitor cells in BMC will not lose their chondrogenic potential as the cultured cells during the *in vitro* monolayer expansion (Huh et al. 2016).

Despite of remarkable potential of BMC, there are also studies that challenge the previous opinions by highlighting the limitation of BMC. Due to the limited volume of chondrogenic niche in BMC, it resulted in lack of stability of the repaired cartilage. The progenitor cells isolated from the density gradient only accounts for 0.001 to 0.01%, however, a high concentration of growth factor including transforming growth factor-beta (TGF-β) and bone morphogenetic proteins 2 and 7 were reported (Chahla et al. 2016).

Prior to the administration of BMC into human use or clinical trials, extensive studies in animal models are essential. Hence, this review is intended to summarise the animal studies that have used the BMC for cartilage repair and regeneration. Limited studies have been done in animal model with the aid of other factors and materials throughout the years and to the best of our knowledge, there is no systematic review focusing on the studies of BMC for the treatment of cartilage defect in animal models. The purpose of this study was to conduct a systematic review focusing on the BMC potential for the treatment of cartilage defect in animal models.

Methods

Search strategy and study design

The online databases used in this study include Scopus, PubMed, and ISI Web of Knowledge. The search terms 'bone marrow concentrate' or 'bone marrow aspirate' and 'cartilage' were used, without any restriction to language and date of publication. These results were searched for studies matching the keywords and 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 2019. The results of the selected articles reviewed here were critically scrutinized based on the treatment outputs such as tissue repair quality and study limitations. Results obtained were scrutinized and screened through to select the most related studies as shown in Figure 1.

Study selection

All literature published up to July 2019, which was related to the usage of BMC alone or with other treatments and materials for cartilage repair in animal models, were selected. Studies were included whether the treatment group received BMC for any cartilage defect compared with another group receiving other cartilage repair treatments. Review papers and other studies that combined effects of BMC for other defects treatments such as bone or meniscus repair were excluded from further analysis.

Data extraction

Data were extracted on: characteristics on selected studies; method of harvesting and processing BMC with the number of cells and delivery methods; quality of repaired tissue (gross morphology, histological, immunohistology and any other tests outcomes where available); limitation of each studies; and the summary outcomes. The outcomes measured involving descriptive data were also considered where possible.

Results

Literature search and selection

The online literature search using PubMed, Scopus, and Web of Science (ISI) found a total of 2474 scientific papers. After excluding duplicates, 1878 were reviewed for suitability and out of that, 1863 were excluded due to unrelated and irrelevant studies. Studies that were included were up to July 2019 and the remaining fifteen studies that speaks exclusively on the cartilage repair using bone marrow concentrate intervention were selected and reviewed in this paper.

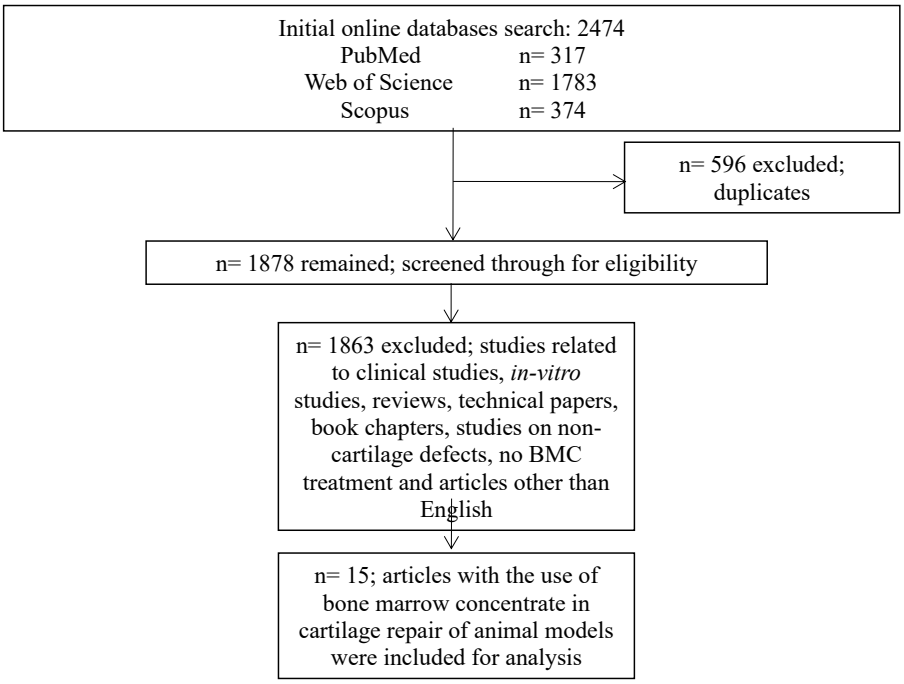

FIGURE 1. Inclusion and exclusion criteria flowchart selection process

CHARACTERISTICS OF SELECTED STUDIES

Studies included for this systemic review described the use of BMC in combination of other factors (EPO, TGF, PRP), techniques (microfracture) and material (HA, PGA, collagen, autologous tissue) to repair cartilage defects in animal models. Out of the fifteen studies; five were using rabbit model, three studies were on mini-pig, two were using horse, two studies on goat, two on sheep model and only one study is using beagle model. Out of fifteen, only two studies have reported the use of BMC independently while comparing with other treatment groups, whilst the other thirteen reports combined the BMC with other treatment methods. Most of the studies have assessed the therapeutic potential of BMC within 2 to 6 months. However, the shortest study period was 1.5 months (Veronesi et al. 2015) and the longest was 12 months (Chu et al. 2018). The thickness of the articular cartilage varies between different species, therefore, depending on the animal species and location of defect, different diameters of defect ranging from 3 to 15 mm and 2 to 10 mm deep were created in the animals. The animals and size of defects created were as follows; three mini-pigs studies with defects sizes between 6 and 7 mm diameter, two goat studies defects were 4 and 5 mm diameter, two sheep studies size of defects were 5.8 and 6.2 mm diameter, five rabbit studies defects made were between 3 and 5 mm diameter, two horse studies with defect size of 15 mm and one beagle study with defect size of 6 mm. The treatment or intervention selected for defects creation also vary between studies. 10 out of 15 studies combined BMC with different types of scaffolds (HA, PGA, Collagen or biphasic) along with factors and cells such as PRP, EPO, and bone marrow-derived MSC (BM-MSC). Four studies were looking into individual or combination effect of microfracture with BMC (Bekkers et al. 2013; Chu et al. 2018; Fortier et al. 2010) and/or autologous osteochondral transplant (Jin et al. 2011); and one study by Ivkovic et al. (2010) was using the growth factor comparing with BMC treated group. Few studies were reported on the complications such as mortality, surgical site infection and replacement of animals during the initial phase of the study.

BONE MARROW HARVESTING TECHNIQUE, OBTAINING CELLS AND DELIVERY METHOD

Most common site of bone marrow aspirate collection reported in these studies was from iliac crest, whilst one study has isolated bone marrow from the sternum Fortier et al. (2010), one from distal femur (Hernigou et al. 2018) and one study did not mention the location of aspirate (Chu et al. 2018). The volume of aspirated bone marrow varies inter and intra species; 3 (Ivkovic et al. 2010) to 20 (Getgood et al. 2012) mL from sheep, 4 mL (Jin et al. 2011; Zhao et al. 2013) to 6 mL (Hernigou et al. 2018; Veronesi et al. 2015) from rabbit, 20 mL from goat (Bekkers et al. 2013), 20 to 24 mL from mini-pig (Betsch et al. 2014, 2013; Jagodzinski et al. 2013) and 60 mL (Chu et al. 2018) to 70 mL from horse (Fortier et al. 2010).

Chu et al. (2018) and Fortier et al. (2010) utilized the SmartPReP® 2 Centrifuge System (Harvest Technology) and Getgood et al. (2012) used Lymphoprep System (Axis-Shield) in processing the BMA. Four studies processed the bone marrow with Ficoll density gradient system (Amersham Biosciences and Sigma) and another four processed with normal centrifugation machines. Bekkers et al. (2013) on the other hand isolated the mononuclear fraction pellet of the centrifuged BMA. Three authors utilized the Marrow Stim Concentration Kit (Biomet Biologics) obtaining 3 to 4 mL of BMC.

Ivkovic et al. (2010) did not process the aspirate and directly mixed it with viral particles for gene modification, and the mixture was allowed to clot at the defect area. The number of cells reported for each study differs in terms of types of cells and volume/concentration as well (Table 1). Nine studies did an open surgery and implanted the scaffold containing the cells directly onto the defect (Betsch et al. 2014, 2013; Getgood et al. 2012; Hernigou et al. 2018; Jagodzinski et al. 2013; Veronesi et al. 2018, 2015; Yoon et al. 2016; Zhao et al. 2013), three studies did an open surgery to directly pipetted or injected cells and scaffolds onto defects (Bekkers et al. 2013; Ivkovic et al. 2010; Jin et al. 2011), two studies delivered the sample using arthroscopy method onto defect (Fortier et al. 2010) and one study utilized the intra-articular injection method (Saw et al. 2009).

COMPARISON OF OUTCOMES BASED ON DIFFERENT METHODS OF TREATMENTS

There were many methods of outcome measured, included but not limited to assessment scores such as International Cartilage Repair Society (ICRS) scoring systems and Rudert scoring system for macroscopic evaluation and MRI, O'Driscoll score and Gill scoring system for histology evaluation. Few studies also included the mechanical testing analysis for the fabricated scaffolds for future improvements (Getgood et al. 2012; Ivkovic et al. 2010; Jagodzinski et al. 2013), TRAP analysis (Jagodzinski et al. 2013), computed tomography testing (Betsch et al. 2014), Micro-CT (Yoon et al. 2016) and magnetic image resonance (MRI) (Chu et al. 2018; Hernigou et al. 2018). Two out of the ten selected studies were by Betsch et al. (2014, 2013) groups and from the thorough analysis of both papers, these studies do not appear to be overlapping each other in terms of method of interventions or the outcomes. Therefore, both papers were included in the analysis. Summary of these outcomes were summarized in Table 2. From the available literature, we have found that up to 90% of the studies hypothesized that BMC provided enhanced repair of the cartilage defect with combination to additional factors, with the exception for studies by Chu et al. (2018) and Getgood et al. (2012). Three studies started off with characterization of the desired cell, MSCs, by using flow cytometry for surface markers detection. In a study by Fortier et al. (2010), the flow cytometry results only at a specific gated area (gate 5) were stained positive for CD 44, CD 29, and CD 172a and negative for both hematopoietic cell markers, CD 34 and CD 45. Studies by Betsch et al. (2014, 2013) groups on the other hand stained positive for CD 44 (> 90%), CD 14 (91.2%), CD 90 (89%), CD 45 (5.9%) and CD 34 (2.5%) in 2013 and positive staining for CD 44 (> 89.3%), CD 14 (93.6%), CD 90 (93%), CD 45 (6.4%), and CD 34 (2.1%) in 2014. The numbers correspond to the percentage stained of all viable single cells.

All of the studies showed that the addition of BMC in combination of other materials enhanced the repair of cartilage tissue compared to the control and other treatment groups, except for studies by Chu et al. (2018) and Getgood et al. (2012). Gross morphology of the repaired tissue gave a variety of outcomes. In treatment groups combining with the BMC, positive outcomes were observed; smoother surface level of defect area with adjacent cartilage, better integration of scaffolds with the surrounding tissue and better filling of the cartilage tissue in the defects. No lesions or inflammations were reported in any of the studies and none of the implanted scaffolds dislodged from the defect area. However, in few of the studies, fibrous cyst (Getgood et al. 2012; Jagodzinski et al. 2013) and subchondral bone cyst (Betsch et al. 2014, 2013) were found in control as well as treatment groups. Details of these findings were summarized in Table 2.

Majority of the studies produced a hyaline or hyaline-like cartilage tissue when combining BMC with additional interventions into their respective treatment

groups. In few studies, the hyaline or hyaline-like cartilage also showed a columnar cluster organization of chondrocytes (Ivkovic et al. 2010). Microscopic view on most control cells observed disordered arrangement of fibrous-like cartilage tissue or mixture of both hyaline- and fibrous-like tissues in some cases. Scar tissues were also visible in most of the control groups. Groups that received any other interventions for defect treatment expressed little to strong staining of collagen type II and/or collagen Type I. Most BMC treatment groups showed intense staining of collagen type II and/or collagen type I. Getgood et al. (2012) was the only study reported that PRP gave the strongest expression of collagen type II with mild collagen type I expression compared to BMC.

Other methods of testing for the quality of repaired cartilage tissue were biomechanical testing of the implanted scaffolds and chondrogenic gene expression of the repaired tissue. In a study by Ivkovic et al. (2010), they found that the BMC treated group gave a similar stiffness of repaired tissue to native cartilage. It was even stiffness was even higher in the modified BMC group (BMC+TGF/BMC+GFP). Jagodzinski et al. (2013) also reported a significant difference in Young's modulus (elasticity) between all BMC treatment groups when compared with control group. Additional TRAP staining was also conducted where no significant differences in number of osteoclasts were found between all groups. Study by Zhao et al. (2013) reported a high expression of three chondrogenic genes; collagen type II, aggrecan and Sox9 mRNAs, in BMC treated group. Micro-CT study by Yoon et al. (2016) showed enhanced subchondral bone regeneration in BMC combination treated group. Magnetic resonance imaging (MRI) were conducted by two studies gave no significant difference between BMC and non-BMC treated groups (Chu et al. 2018; Hernigou et al. 2018). Details of the findings were summarized in Table 2.

TABLE 1. BM harvesting technique, BMC processing method, number and types of cells and the outcome

Author	BM harvesting & concentrating	Types & number of cells (mean/range)	Cell delivery method
Saw et al. (2009)	Bone marrow harvested from bilateral iliac crest and centrifuged at 1,900 rpm for 10 min	Mean total nucleated cell, 220×10^6 cells and range from 159 to 438×10^6 cells	Intra-articular injections
Fortier et al. (2010)	~ 60 mL of bone marrow harvested from the sternum and concentrated using SmartPReP® 2 Centrifuge (Harvest Technologies), obtaining 6 mL BMC	-	Arthroscopy, directly injected onto defect
Ivkovic et al. (2010)	3 mL of bone marrow harvested from right iliac crest	-	Open surgery, directly pipetted onto defect
Jin et al. (2011)	~ 4 mL of bone marrow harvested from iliac crest and concentrated using Ficoll gradient centrifugation system, obtaining 20 µL of BMC	MNC: $9.2 \pm 3.9 \times 10^6$ cells and MSCs: 607.8 ± 175.98 cells/mL	Open surgery, directly injected onto defect
Getgood et al. (2010)	~ 20 mL of bone marrow harvested from the posterior iliac crests density gradient medium and concentrated by dilution with 20 mL of PBS and layered onto Lymphoprep (Axis-Shield, Oslo, Norway), centrifuged at 900 g for 20 min. Buffy layer was removed, and a cell pellet produced *via* further centrifugation at 750 g for 10 min	Total nucleated cell: 0.25 to 6.87×10^9/L MNC: 0.1 to 3.45×10^9/L	Open surgery, contained in scaffold
Zhao et al. (2013)	~ 4 mL bone marrow samples from iliac crest were concentrated by centrifugation in a Ficoll gradient (Sigma) at 1,500 rpm for 10 min, obtaining 40 µL of concentrate	Average number of: MSC in BMS: 26.3 ± 6.0 MSCs/40 µL MSC in HIC: 27.6 ± 6.5 MSCs/40 µL	Open surgery, contained in scaffold

Bekkers et al. (2013)	~ 20 mL bone marrow harvested from the iliac crest and concentrated at 300 g for 10 min and cell pellet collected and diluted in red blood cell lysis buffer (Sigma, The Netherlands). Cells spun down and wash with PBS, producing MNF fraction	-	Open-surgery, direct injected onto defect
Betsch et al. (2013)	~ 24 mL of bone marrow harvested from the iliac crest and concentrated using point-of-care device (MarrowStim mini concentration system, Biomet Biologics, Inc., USA), obtaining 3-4 mL BMC	Mean volume of MNC in: a) BMAC: 23.08 × 10^6 cells/mL ± 24.12 b) PRP+BMAC: 43.85 × 10^6 cells/mL ± 43.00	Open surgery, contained in scaffold
Jagodzinski et al. (2013)	~ 20 mL of bone marrow harvested from dorsal aspect of the posterior iliac crest and advanced into the intra-medullary cavity and concentrated using concentration system (Marrowstim, Biomed, Warsaw, Indiana), obtaining 3 mL BMC	Mean of nucleated cells in BMAC 10.7 ± 2.6 million/mL	Open surgery, contained in scaffold
Betsch et al. (2014)	~ 24 mL of bone marrow harvested from the iliac crest and concentrated using point-of-care device (MarrowStim mini concentration system, Biomet Biologics, Inc., USA), obtaining 3-4 mL BMC	Mean ± SD of MNC in BMC: a) BMAC 95.18 × 10^6 cells/mL ±61.28 b) EPO+BMAC 97.66 × 10^6 cells/mL ± 64.20	Open surgery, contained in scaffold
Veronesi et al. (2015)	6.0 ± 1.5 mL of bone marrow harvested from posterior iliac crest of each animal and concentrated using Ficoll-Paque (density 1.083 g/mL) (Sigma-Aldrich, Milan Italy) centrifugation system at 600 g for 30 min	2.03 × 10^6 bone marrow mononuclear cells	Open surgery; contained in scaffold
Yoon et al. (2016)	24 mL of bone marrow aspirate harvested from iliac crest and centrifuged for 15 min at 2977 g, and red blood cells were then selectively lysed using the ACK lysing buffer (Gibco), obtaining 0.3 mL of BMC	-	Open surgery, contained in scaffold
Chu et al. (2018)	60 mL of BMA harvested and processed using SmartPReP® 2 BMAC (Harvest Technologies) according to the manufacturer's protocol	Centrifugation Mean total MNC: 9.66 × 10^9 ± 2.37 × 10^9 Ficoll gradient Mean total MNC: 7.32 × 10^8 ± 3.35 × 10^8	Arthroscopy; BMC clotted using thrombin and applied directly onto defect
Hernigou et al. (2018)	6 mL bone marrow sample harvested from internal condyle of the contralateral distal femur and layered onto 4 mL Ficoll-Hypaque solution (1.077 g/cm^3), undergo double centrifugation; obtaining BMAC pellet dissolved with 0.25 mL sterile PBS	Mean concentration of mononuclear cells: 21 × 10^6 cells/mL	Open surgery, contained in scaffold
Veronesi et al. (2018)	5.0 ± 0.5 mL bone marrow aspirate harvested from posterior iliac crest of each animal and centrifuged 1000 rpm for 10 min	-	Open surgery, contained in scaffold

[a] BM, bone marrow; BMA, bone marrow aspirate; BMAC, bone marrow aspirate concentrate; EPO, erythropoietin; MNF, mononuclear fraction; PBS, phosphate buffer saline, BMS, bone marrow stimulation, MSC, mesenchymal stem cell; HIC, harvested iliac crest; PRP, plasma rich protein

TABLE 2. Comparison of outcomes based on different methods (according to group of treatments)

Author	Gross morphology	Histology	Immunohistology	Other testing	Outcomes
Saw et al. (2009)	a) Control & b) HA: semi-transparent tissue, recognizable margins, irregular surface b) HA+BMA: coverage almost complete, surfaces smooth and level with normal cartilage	a) Control: scar tissue present, disordered arrangement of fibroblasts, proteoglycans absent b) HA: less visible scar tissue, hyaline-like cartilage at interface with subchondral bone and adjacent to normal cartilage at the defect margins, proteoglycans only at base and sides of defect in the same distribution as hyaline like cartilage c) HA+BMA: chondrogenesis with hyaline cartilage formation, proteoglycan accumulation in the deeper layers	a) Control: resence of collagen type I and absence for collagen type II b) HA: type I collagen less visible, light staining type II collagen around areas of hyaline-like cartilage c) HA+BMA: type I collagen staining found only in the perichondrium deeper cartilage stained strongly for type II collagen	-	Intra-articular injections HA with BMA gave better cartilage repair, assessed histologically
Fortier et al. (2010)	BMC+MF: thicker, more hyaline-appearing repair tissue and better integrated MF: full-thickness fissures radiating from the periphery of the defect and the lack of fill in the proximal-medial ¼ of the defect were still visible	Increased proteoglycan staining primarily located in the deeper layers than superficial layers of the repair tissue of BMC+MF compared to MF	Type-II collagen expressed greater in BMC+MF group than in MF group. Bottom 50% or more of the repair tissue positive for type-II collagen in BMC+MF group but only the very lowest layer expressed type-II collagen in MF	Second look arthroscopy: BMC+MF had significantly higher ICRS score than MF alone (6.9 ± 1.3 vs. 3.2 ± 0.9; p = 0.002)	BMC+MF result in healing of acute full-thickness cartilage defects that is superior to MF alone
Ivkovic et al. (2010)	-	a) No treatment: acellular tissue within the defect with intact calcified layer and subchondral bone b) BMClot: defect fill with fibrocartilage, clear demarcation between native hyaline and fibrocartilage, separated by a defect gap	GAG analysis: no significant difference between mean values of repaired cartilage in the treatment groups and native cartilage from contra-lateral knees	Biomechanical testing: BMC group has very similar stiffness to native cartilage. Stiffness higher in TGF and GFP groups	Genetically modified bone marrow clots are sufficient to facilitate articular cartilage repair of partial thickness defects *in vivo*

		c) GFP: irregular filling, mixture of hyaline and fibrocartilage, clusters of clonal cell division present in upper layers d) TGF: hyaline cartilage and columnar organization of chondrocytes detected on both sides of the defect gap	Collagen type I: significantly higher in all treatment groups compared with native cartilage, but content in BMC significantly lower from than in GFP and TGF groups Collagen type II: significantly lower in BMC and GFP treatment groups when compared with native cartilage	Stiffness significantly higher in GFP group than in TGF group whereas BMC had lower compared with both GFP and TGF	
Jin et al. (2011)	All filled with repaired tissue except AOTS group	a) No treatment: fibrous tissue b) AOTS: hyaline cartilage, no degeneration, graft tissue not integrated c) BMS: fibrous-like tissue d) BMS+BMC: well organized, intense ECM, columnar cluster cells, hyaline tissue	GAG in BMS+BMC significantly higher than in control and BMS groups, but not as much as in the AOTS and Normal groups No statistically significant difference in the amount of GAG in the Buffy coat group, AOTS group, and normal cartilage	-	BMS+BMC cartilage repair better than BMS alone
Getgood et al. (2012)	Increase in ICRS score with the scaffold + PRP and scaffold + CBMA groups compared to the empty defects and scaffold alone, but no statistically significant difference existed between groups	Increased O'Driscoll score noted in the scaffold +PRP group compared to the other 3 treatment groups, particularly in the MFC, not statistically significant a) Empty defect, scaffold and scaffold + CBMA defects: thickness of cartilage repair tissue less than thickness of normal adjacent cartilage. b) scaffold + PRP defects: cartilage thickness restored to nearly normal	a) Empty defect: type I collagen staining with no pericellular type VI collagen staining and little type II collagen staining, indicating a fibrocartilage tissue b) scaffold only: both type I and II collagen staining with pericellular type VI staining in the lateral margins, indicating	Biomechanical testing: All treatment groups in the MFC and LTS found to have similar mean stiffness measurements compared to the contralateral limb and perilesional cartilage	Scaffold + PRP produce repair tissue with more characteristics of hyaline cartilage. CBMA combined with collagen-GAG biphasic scaffold shows no benefit over an empty 5.8-mm osteochondral defect or a defect filled with scaffold alone apart from a reduction in subchondral cyst formation

		Reduction at both sides in appearance of residual scaffold in scaffold + CBMA group compared to other groups. Cysts only detected in control and control + scaffold groups; 22% of sections from the empty or scaffold only groups had cysts compared to none in scaffold + PRP or scaffold + CBMA groups a) Empty defects: proteoglycan staining in repair tissue in all MFC empty defects and relatively poor staining in the LTS empty defects b) Scaffold only and scaffold + CBMA: moderate staining in all sections c) Scaffold + PRP sections: strong staining, indicating high proteoglycan content within the repair tissue	a mixed hyaline/ fibrocartilage repair c) scaffold+CBMA: both LTS and MFC showed good type II collagen staining and reduced type I collagen staining compared to the scaffold only treatments, with pericellular type VI collagen present at the margins of the MFC defect d) Scaffold + PRP defects: strong type II collagen staining with mild type I staining. In MFC defect, pericellular type VI collagen detected throughout the repair zone	However, there was a trend toward increasing stiffness seen in empty defects in both LTS and MFC	
Zhao et al. (2013)	a) untreated group: partially empty or concave, and some regenerated tissue observed in peripheral regions b) BMS and PGA: filled with whitish repaired tissue that appeared distinguishable from surrounding cartilage c) BMC: filled with glossy white repaired tissue appeared to be smooth and well-integrated with	a) untreated: faint toluidine blue staining observed in margin areas adjacent to native cartilage b) BMS: mixture of fibrous tissue and cartilage-like tissue, as shown by HE and toluidine blue staining but graft tissue not integrated with host tissue c) PGA: relatively smooth surface, weak toluidine blue staining of regenerated fibrous-like tissue d) BMC: filled with well-organized tissue, regular surface and well-integrated with native cartilage. Composed of columnar and cluster cells with hyaline character	a) Type II collagen staining: stronger, smoother, and more regular in BMC group than the first 3 groups b) Type II collagen staining: each group essentially equivalent to toluidine blue staining regions. Intensively stained collagen tissues discovered in both BMC and BME groups	Lower DNA content detected in BMC group than in other groups, and no significant difference between PGA and BMS groups	Combination of BMC and PGA scaffold can supplement BMS in rabbit articular cartilage repair.

	surrounding tissues, remained slightly concave in the centre d) BME: smooth and whitish, repairing effect similar to BMC group in the macroscopic view	e) BME: plenty of chondro-like cells and ECM, integration between implanted tissue and host tissue observed, although not so smooth	c) GAG: BMC group significantly higher than in the first three groups, and no statistically significant difference between amounts of GAG in PGA and BMS groups	Real-time PCR: revealed that levels of collagen II, aggrecan and Sox9 mRNAs, the three chondrogenic genes, were higher in both BMS and HIC groups than in control group. No differences in chondrogenic gene expression between BMS and HIC groups	
Bekkers et al. (2013)	MF group showed less defect fill at 6 months compared to chondrocyteMNF group No differences in scores between the two groups of the femur cartilage surface Post-treatment macroscopic scores of the articulating tibia cartilage statistically significantly higher in MF-treated defects compared to chondrocyteMNF treated defects	Microscopic view of regenerated tissue in defects treated with chondrocyteMNF appeared better compared to microfracture-treated defects although still incomplete. Defect fill present after chondrocyteMNF treatment O'Driscoll score for chondrocyteMNF-treated defects appeared to be higher compared to microfracture-treated defects, but shows no statistical significance	GAG production per gram of regenerated tissue did not show statistically significant differences between two treatments (25.61 ± 14.95 mg GAG per gram tissue vs 23.51 ± 6.82 mg GAG per gram tissue for MF and chondrocyteMNF)	-	Treatment using a combination of MNF cells from bone marrow and unexpanded chondrocytes leads to statistical significantly higher macroscopic regeneration scores compared to MF
Betsch et al. (2013)	No lesions on corresponding articular surfaces and no inflammation of synovial membrane. None of the 28 implanted scaffolds dislodged	Remnants of implanted scaffolds consistently present in osseous phase of the defects, while cartilage phase completely replaced after 26 weeks.	Newly formed tissue in cartilage area of defects in therapy groups stained blue with toluidine blue and contained collagen II based on positive immunostaining	-	Addition of PRP or BMAC to biphasic scaffold led to better healing of osteochondral defects compared control group, but combination of both therapies did not further enhance healing

		Repair tissue in bony phase fibrous with vascularisation and giant cells, indicating on-going degradation process of scaffold at 26 weeks. In 10 out of 28 defects, subchondral cyst noted in subchondral layer	Mostly fibrous tissue found in defects of control group, which did not stain for collagen type II or sulphated glycosaminoglycans (sGAG)	-	
Jagodzinski et al. (2013)	a) Empty defect: 4/5 healed with remaining fibrous cyst without trabecular bone b) Upside-down cylinders: remaining bone defect found in 3/5 animals c) Scaffold & Scaffold+BMCC: defects healed with reconstruction of underlying subchondral bone plate	a) Empty defect: Gradual change from central fibrous tissue to fibrocartilage in 3/5 specimens and no transition zone into surrounding healthy hyaline cartilage b) Upside-down cylinders: Hyaline cartilage formation found on overgrowing edges of defect c) Scaffold & Scaffold+BMCC: transition zone from the regenerate with varying amounts of fibrocartilage into surrounding hyaline cartilage	Positive staining for type II collagen in Scaffold & Scaffold + BMCC groups when compared with negative stained controls	TRAP staining: no significant difference in number of osteoclasts between groups Mechanical testing: significant difference in Young´s modulus between all experimental groups and controls	Filling of osteochondral defects with porous scaffold and with addition of BMCC decreases defect size compared with autologous spongious bone cylinder or if defects are left empty
Betsch et al. (2014)	i) No abrasions on opposing articulating surfaces ii) No inflammation of synovial membrane or other joint tissues. iii) No dislodged scaffolds iv) Repaired tissue well integrated with native cartilage in therapy groups	a) Therapy groups: positive staining of toluidine blue for chondrogenic tissue b) Control: repaired tissue generally fibrous, deficient in sGAG c) Residual scaffolds consistently found in osseous phase of defects after 26 weeks. ECM of repair tissue in bony phase was vascularised but disorganized, occurrence of foreign-body giant cells, indicates that process of scaffold degradation still on going after 26 weeks. In 9 defects, subchondral bone cyst found in area around the incompletely resorbed scaffold	a) Therapy groups: positive staining for collagen II b) Control: fibrous tissue, deficient in collagen type II	Cone-beam computed tomography of a representative medial femoral condyle delineated a cystic radiolucent area, which smaller than originally cylindrical repair area, indicating that bone adjacent to implanted scaffold began to remodel starting from edges of defect	EPO+BMAC enhanced osteochondral healing

Veronesi et al. (2015)	a) Scaffold only: Partially empty, rough brown fibrous tissue, slightly empty in the centre b) Scaffold/ BMC: Translucent with high degree of filling and integration c) Scaffold/PEMF: Irregularity in well-integrated surface, filled up to adjacent tissue e) Scaffold/BMC/ PEMF fully filled with integrated and transparent cartilage-like tissue (indistinguishable from normal adjacent tissue)	a) Scaffold only: Fibrocartilage and poor GAG staining with altered cell distribution b) Scaffold/BMC: Fibrocartilage with normal cell distribution c) Scaffold/PEMF: Mix of hyaline and fibrocartilage, moderate matrix staining, clusters of chondrocytes in few zones d) Scaffold/BMC/PEMF: Hyaline cartilage with normal GAG content, smooth surface and normal chondrocytes distributions. Bone reconstruction complete	-	-	Scaffold/BMC/ PEMF group gave better outcome for osteochondral regeneration
Yoon et al. (2016)	a) IL8-scaffold, BMC-scaffold & MSC-scaffold: Incomplete regeneration but better than PBS-scaffold group c) IL8/BMC-scaffold: Defect almost entirely regenerated	H&E Staining: IL-8/ BMC group showed chondrocyte-like cells with smooth cartilage-like tissue compared to other groups MT Staining: Higher collagen content in IL-8/ BMC group Safranin-O Staining: Increase in GAG synthesis in IL-8/BMC and IL-8 groups	Only IL-8/BMC group induced the expression of type II collagen and aggrecan on regenerated cartilage	Micro-CT: Subchondral bone regeneration enhanced in IL-8 and IL-8/BMC groups compared to the control	Combination of IL-8 and BMC gave a significant enhanced impact on osteochondral regeneration
Chu et al. (2018)	Arthroscopy morphology BMC: 6 of 7 horses have 50% defect filled Microfracture: 5 of 7 horses have 50% defect filled	Microfracture and BMC group both gave a similar in fibrous tissue regeneration. No significant difference between ICRS scores of both group	-	Morphological & Quantitative MRI: BMC group gave better tissue regeneration (4 of 7 horses) than Microfracture (2 of 7 horses), no significant differences between both groups	BMC application gave a similar outcome to microfracture on critical-sized defect

Hernigou et al. (2018)	a) Control: Defect visible, filled with repaired white tissue and nearly completely present at defect edge b) Scaffold & Scaffold/BMMC: Both edge and bottom of defect totally filled with white hard and translucent soft tissue; close to normal tissue level	All defects fully filled with cartilage tissue	Control and treatment groups showed heterogeneity of tissue organization with total or partial differentiation in hyaline cartilage, with few producing heterogenicity of fibroblastic and partially differentiated hyaline cartilage	MRI: All groups defect entirely filled with comparable thickness and signal of the normal cartilage surroundings	Combination of scaffold with BMMC gave a better outcome in tissue regeneration of cartilage lesion
Veronesi et al. (2018)		3 months: All groups except for SC + BMC showed fibrous-tissue and no hyaline-like cartilage regeneration Formation of subchondral bone in both BMC's groups 6 months: SC+BMC showed hyaline cartilage formation while SC + SN-BMC showed greater cartilage organization	3 months: Untreated defect group showed higher collagen I expression compared to other groups 6 months: Untreated defect group showed higher collagen I expression compared to other groups Collagen II highly expressed in SC + BMC group compared to untreated defect	-	BMC combination groups gave most successful repair potential for osteochondral defect

[a]ICRS, International Cartilage Repair Society; AOTS, autologous osteochondral transplantation; MF, microfracture; GFP, green fluorescent protein; TGF, transforming growth factor; AOTS, autologous osteochondral transplantation; ECM, extracellular matrix; BMC/BMCC/BMAC/; CBMA, concentrated bone marrow aspirate; BMS, bone marrow stimulation; GAG, glycosaminoglycan; FACS, Fluorescence-activated cell sorting; EPO, erythropoietin; SC, scaffold; SN, surnatants; MRI, magnetic resonance imaging; HA, hyaluronic acid; BMClot, bone marrow clot; BME, BMS together with composite of PGA and cultured bone marrow stem cells ; ChondrocyteMNF, chondrocyte mononuclear fraction; BMCC, bone marrow derived cell concentrate; EPO, erythropoietin; PEMF, pulse electromagnetic field

TABLE 3. Limitations and future study suggestions (as observed and mentioned in the publication)

Studies	Limitations and future study suggestions
Saw et al. (2009)	a) Small sample size (n = 15) with the addition of animal death b) No radiographic documentation of skeletal maturity c) Lubricin and type X collagen staining with biomechanical testing of repaired tissue for high-quality hyaline cartilage should be done d) Challenges in controlling post-surgery movement of animals i.e. passive motion and avoidance of weight bearing e) Validation of results in skeletally mature animals with MA injections alone should be done f) Temporal progression study of repair process should be considered i.e. 1,2 and 4 months after surgery
Fortier et al. (2010)	a) Longer study period required for evaluation of durability of repaired tissue b) Acute cartilage defects were made in healthy joints and that does not reflect the relevance in human clinical diagnostics c) No quantification of growth factors which could show strong correlation platelets and anabolic growth factors such as PDGF and TGF-b in platelet-rich plasma d) Quantification of the exact number of cells in BMC necessary in relation to the quality of repaired tissue
Ivkovic et al. (2010)	a) Usage of a single factor to stimulate and regulate chondrogenic differentiation might limit the production of optimal quality b) Challenges in controlling post-surgery movement of animals
Jin et al. (2011)	a) No comparison of BMC group only to other treatment groups to study if the number of cells from BMC was overwhelmed by the microfracture
Getgood et al. (2012)	a) Variability in PRP obtained for application onto the scaffold b) Small defect size which could be crucial to the repaired tissue and perilesional cartilage c) Longer period of study needed to assess complete tissue healing for accurate comparison between groups
Zhao et al. (2013)	a) No control group using another scaffold for comparison purposes b) No growth factors added for the proliferation and chondrogenic differentiation of MSCs
Bekkers et al. (2013)	a) Incomplete defects fill in this study could have contributed to the slight to moderate degeneration seen after 6 months follow-up in treatments b) Bilateral approach could also have added to the slight degeneration observed in both treatments at distant locations in the joint. This is important as most treatment failures or insufficient clinical improvement after cartilage therapy can be brought down to inadequate defect fill and tissue regeneration c) Longer follow-up observation, up to 2 years should be considered for more definite and objective conclusions
Betsch et al. (2013)	a) Evaluation was done for osteochondral repair in acute, but not chronic mini-pig model. However, in clinical cases, human defects are usually of chronic nature. These differences should be considered when translating the findings in clinical settings
Jagodzinski et al. (2013)	a) Longer follow-up of one year would have been desirable. Differences between the groups were small and are likely to have become smaller during further follow-up b) Power of study should be done in determining the optimal sample sizes c) Further characterization of heterogenic constructs on molecular level creates difficulties due to the wide range of regenerate quality within the defects d) Limited value for clinical transfer due to model limitations and the surrounding tissue is mostly less favourable in a pathologic osteochondral defect e) Defined cell numbers with different cell phenotypes are recommended for future studies

Betsch et al. (2014)	a) Longer evaluation period needed as biphasic scaffold takes up to two years to fully resorb b) Immediate weight-bearing after surgery could have impeded with early phases of bone and cartilage regeneration and weight-bearing restrictions in large animal models are challenging c) Imaging techniques such as MRI could be useful in addition to a histological score, by further characterize and quantify the repair tissue
Veronesi et al. (2015)	a) Short-term study and single experiment do not allow cartilage to mature and progressive deterioration of repaired tissue to be observed b) Mechanical studies to justify the PEMF effects need to be done
Yoon et al. (2016)	a) BMC prepared in the final stage was filtered and MSCs and HSCs were removed. Therefore, the BMC only group does not give any therapeutic effect on the defect b) Only a preliminary study for IL-8/BMC combination and have yet been confirmed in most species where more studies need to be conducted
Chu et al. (2018)	a) Sample size is too small (n = 8) to confirm the findings effectiveness
Hernigou et al. (2018)	a) Spontaneous healing of osteochondral defect for non-skeletal mature rabbits might contribute to the better healing b) Differences in scoring system that differs greatly based on specific topics (cartilage only; cartilage and subchondral bone; biomaterial) c) Anatomy and biochemical of rabbit's cartilage differ from human (thinner hyaline cartilage layer)
Veronesi et al. (2018)	a) Cellular and molecular mechanism affected by the exposure of paracrine factors to stress environment during surgery. Need options for future treatments modalities

[a]BMC, bone marrow concentrate; MA, marrow aspirate; HA, hyaluronic acid; MF, microfracture, MSC, mesenchymal stem cell; PRP, plasma rich protein; MNF, mononuclear fraction; MRI, Magnetic resonance imaging; IL-8, interleukin-8; PEMF, pulse electromagnetic field

DISCUSSION

The major findings from the published studies in the selected articles that has been included this review do not appear to unanimously agree that the application of BMC provides the best repair outcomes for cartilage defect, ranging from good to excellent, compared to other intervention method. Although results and findings of these studies appear to be valid, there are very limited number of published animal studies and data relating to this subject matter (only 15 studies or publications were found to be relevant).

The analysed studies demonstrated a good effect for BMC in treating osteochondral defects, specifically the cartilage defects itself, most of which between 4 and 15 mm diameter and up to 10 mm deep. In the selected studies, BMC was used together with microfracture, microfracture, and scaffold or other additional factors. Most of the available animal studies have reported positive outcomes of using BMC in treating cartilage defect. The known composition of BMC are MSCs, hematopoietic stem cells, platelets, platelet derived growth factors and cytokines. In addition to that, the presence of anti-inflammatory and immune-modulatory properties of bone marrow stem cells can also enhance tissue regeneration. MSCs present in the BMC is said to be the main player in improving the quality of cartilage repaired by increasing the aggrecan content and firmness of tissue (Sampson et al. 2013).

Even with the promising potential of BMC, the best isolation method to optimal concentration of BMC and the best possible way to deliver the BMC has yet to be reported. In addition to that, the components in BMC which are responsible to obtain the optimum cartilage repair remain a question. From the literature, it was understood that the percentage of progenitor cells in BMC, which is the MSCs, are very limited (Chahla et al. 2016) and it varies depending on the location and volume of harvested cells. The optimal number of nucleated cells injected or transferred coupled with the number of administrated dosages to the defects remains unanswered since all

selected studies are using different animal models with varying defect sizes, locations, and treatments. Only a study by Saw et al. (2009) administered three injections of BMC and HA monthly to the treatment groups while the remaining fourteen studies were only administered once. The defect treatment outcomes gave almost the same outcome, good scar tissue recovery and the formation of hyaline cartilage for either once or three times of BMAC administered. A paper by Murphy et al. (2015) discussed on the importance of technique of bone marrow harvesting as the most important aspect in determining the optimum MSC concentration. The authors mentioned that large aspiration volumes from a single site give a significantly lower MSC concentration while smaller aspirates volume increased the nucleated cell counts and the CFU-F frequency of the nucleated cells (Murphy et al. 2015).

These limitations gave opportunity for more studies to be conducted to find the best possible method. This will then be able to close the gap between the current basic science knowledge and the application of it in clinical studies. Understanding the limitations of each study is important for future improvement by developing and modifying current methods in the hope to produce better outcomes.

Determining the optimal sample size is very important to obtain the best statistically possible outcomes of any study. Small number of samples may possible be one of the main limitations in most, if not all reviewed studies. In addition to that, the death or removal of infected or injured animals lowered the sample size that could possibly contribute to the insignificant statistical outcomes (Bekkers et al. 2013; Fortier et al. 2010; Getgood et al. 2012; Saw et al. 2009). Skeletal maturity study could also be done to match to the human skeletal matured system, to shorten the gap from animal study to clinical study (Saw et al. 2009). The challenges of using smaller animal models compared to larger animals, such as rabbits and mini pigs, with thinner layer of cartilage tissue with smaller defect size could induce spontaneous intrinsic healing, which findings could not be represented accordingly (Hernigou et al. 2018; Yoon et al. 2016).

Other than that, biomechanical study is also important to demonstrate that the repair tissue is of a high-quality hyaline cartilage which could withhold weight loading of the animals and eventually to translate it in human study. It was physically impossible to control the motion of animals after surgery to mimic human recovery period after the surgery, especially for quadrupeds which will naturally offload the operated knee until the pain is gone (Saw et al. 2009). Immediate weight bearing also might impeded the early phase of bone and cartilage regeneration and technically challenging and final proof of its efficacy in the cartilage repair of animals is still lacking (Betsch et al. 2014).

Only few studies from the selected papers conducted imaging analysis such as MRI and Micro-CT, which could benefit in giving additional information on the regeneration potential of osteochondral defect using the BMC combination treatment without being invasive. MRI can be used to observe the thickness of repaired cartilage in comparison to the adjacent normal cartilage. Apart from that, the integration of the repaired cartilage into its surroundings and its adhesion to the subchondral bone can also be observed (Chu et al. 2018; Henrigou et al. 2018). Micro-CT on the other hand focused more on analysing the subchondral bone layer condition of the repaired defect, the density of the underlying bone of the cartilage, and even abnormalities formed such as bone cyst/overgrowth formation (Yoon et al. 2016).

Short study period too, might not permit full observation for complete recovery of the defects, which therefore, might not give the most accurate outcomes comparable to studies that were conducted longer (Bekkers et al. 2014, 2013; Fortier et al. 2010; Jagodzinski et al. 2013). Variability in the sizes and locations of the defects according to the species could be an important factor for the tissue regeneration rate. If the defect were done on a non-load bearing area, the outcomes might be better compared to the one location at the weight-bearing area. Therefore, it could not give a clear outcome on the possibility of the knowledge transfer in clinical studies. The scoring systems of the treated tissue also vary between studies and it might have not reflected the actual comparison between studies and treatments. Details of the limitations were summarized in Table 3.

We recognize that this systematic review has its limitations. For the objectives of this review, major limitation of most studies was BMC was not used as a single mean of treatment comparing to other interventions. More studies on using BMC alone might have provided better understanding the use of BMC alone in cartilage repair and its superiority over other treatment methods, if any. For all animal models, evaluation was done in terms of the repair quality of the acute injury, not the chronic injury, which in most human cases chronic injury was more common. The defects were also created on healthy joints, which are not always the case in human injuries,

which do not translate the real clinical symptoms. Due to the animal model limitations, such as different gait, weight-bearing capacity and weight-loading mechanism than human, the value for clinical transfer of the data are limited. Therefore, there is a gap which needs to be filled to ensure that in the future, these animal studies could be a platform of which the knowledge could be transferred to the clinical studies (Table 4).

TABLE 4. Summary from the systematic review

Main point	Details
BMC harvesting techniques	BMC isolation site: mainly from iliac crest
	BMC volume harvested: varies between species; larger animal model provides more volume compared to smaller animal models
	Repair period: 2 to 6 months
	Defect size and thickness: varies between species; larger animal models have larger diameter and depth compared to smaller animal models
BMC processing methods	Majority of the studies utilized commercialized kit in preparing the BMAC that adapted the centrifugation-based method
Cells types used	Majority of the studies reported the number of mononuclear cells from the BMAC isolated, with no or less available data on mesenchymal stem cells or hematopoietic stem cells
Cells delivery method	Majority of the studies used the open surgery method
Treatment groups	Majority of the studies combined BMAC with scaffolds
Outcomes	Majority of the studies gave favorable outcomes in terms of the regeneration and quality of the repaired tissue:
	Hyaline or hyaline-like cartilage formation was observed
	Gross-morphology: high scoring for the repaired tissue
	Strong Collagen type II staining
Limitations	Majority studies gave back the same limitations which are: long-term treatment period needed for observing complete tissue repair

[a] BM, bone marrow; BMA, bone marrow concentrate; BMAC, bone marrow aspirate concentrate

CONCLUSION

From this systematic review, the findings showed that bone marrow concentrate has potential in providing cartilage repair either alone or in combinations. Due to limitations of the currently available studies, the usage of larger animal models with uniformity in the defects created and the tests applied, concrete outcomes for cartilage repair and regeneration will able to be achieved. This will reduce the gap between the current applications of basic science in animal studies with the future clinical application of a one-step cartilage repair procedure in human.

ACKNOWLEDGEMENTS

The study was supported by the Malaysian Ministry of Higher Education, Transdisciplinary Research Grant Scheme (Grant No. TRGS/1/2016/UM/01/4/1).

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Received: 21 February 2020
Accepted: 8 October 2020