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RESEARCH

Stem cell therapy for enhancement of bone consolidation in distraction osteogenesis

A CONTEMPORARY REVIEW OF EXPERIMENTAL STUDIES

Y. Yang,
S. Lin,
B. Wang,
W. Gu,
G. Li

Changzhou No.7 People's Hospital and The Chinese University of Hong Kong, China

Objectives

Distraction osteogenesis (DO) mobilises bone regenerative potential and avoids the complications of other treatments such as bone graft. The major disadvantage of DO is the length of time required for bone consolidation. Mesenchymal stem cells (MSCs) have been used to promote bone formation with some good results.

Methods

We hereby review the published literature on the use of MSCs in promoting bone consolidation during DO.

Results

Studies differed in animal type (mice, rabbit, dog, sheep), bone type (femur, tibia, skull), DO protocols and cell transplantation methods.

Conclusion

The majority of studies reported that the transplantation of MSCs enhanced bone consolidation or formation in DO. Many questions relating to animal model, DO protocol and cell transplantation regime remain to be further investigated. Clinical trials are needed to test and confirm these findings from animal studies.

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Article focus

- Distraction osteogenesis (DO) harnesses the bone regenerative potential of bone and avoids the complications of other treatments such as bone grafting. The major disadvantage of DO is the length of time required for bone consolidation. Mesenchymal stem cells (MSCs) have been used to promote bone formation in this phase, with some good results. This review focuses on the use of MSCs in promoting bone consolidation during DO. Studies differed in animal type (mice, rabbit, dog, sheep), bone type (femur, tibia, skull), DO protocols and cell transplantation methods. Most studies reported that the transplantation of MSCs enhanced bone consolidation or formation in DO. Many questions relating to the animal model, DO protocol and cell transplantation regime require further investigation. A consensus is required on this before further research is undertaken. Clinical trials are needed to test and confirm these findings once a consensus has been agreed on the protocols involving animal studies.

Key messages

- The length of time required for bone consolidation remains a problem in distraction osteogenesis (DO);
- Mesenchymal stem cells can migrate to the sites of injury and stimulate bone regeneration. This may be used to accelerate bone consolidation in DO;
- Despite the variation in animal model, cell source, cell number, administration method, treatment time-point and outcome assessment, MSCs proved effective in regenerating bone during DO.

Strengths and limitations

- Strengths: three tables are used to display the animal models, outcomes, DO protocols and characteristics of MSCs which have been reported in the literature.

■ Y. Yang, MSc, Director, Department of Key Laboratory,
■ W. Gu, MD, Dean, Department of Traumatology, Changzhou No.7 People's Hospital, No. 288 Yanling East Road, Changzhou, Jiangsu, China.
■ S. Lin, PhD, Postdoctoral Fellow,
■ B. Wang, MSc, Postgraduate student,
■ G. Li, PhD, MD, Professor, Department of Orthopaedics and Traumatology, Lui Che Woo Institute of Innovative Medicine, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Prince of Wales Hospital, Shatin, NT, Hong Kong, China.

Correspondence should be sent to G. Li; email: gangli@cuhk.edu.hk

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- Limitations: with the eligibility criteria, the number of selected publications is only 16. This is too small to come to a convincing conclusion.

Introduction

Bone can repair and regenerate itself following damage, but not in the case of significant/segmental bone loss, when surgery with bone grafting or transplantation is usually required. The goal in bone defect management is to align the bone segments, facilitate union, maintain or obtain equal limb length, and restore the function of the limb. Many factors can influence the outcomes, and many alternative therapies have been suggested.[1,2]

The distraction osteogenesis (DO) technique is frequently employed to manage large bone defects.[3,4] DO is widely applied in the treatment of firearm injuries, deformities, nonunion, lower-limb malignant metaphyseal bone tumours or tumour resection.[5-9] Large defects or growth deficiencies severely compromise patients' limb function and quality of life, and their treatment is a challenge.[10] Compared with the conventional techniques for bone reconstruction, DO is a simpler procedure that has a shorter operation time, less blood loss, a shorter duration of hospitalisation, lower cost and risk of complications.[11-13] The DO procedure involves three sequential phases: latency; active distraction or lengthening; and consolidation.[14,15] It stimulates new bone formation through the controlled separation of two osteogenic fronts and also promotes the regeneration of the surrounding soft tissue.[16,17] DO resembles an *in vivo* form of tissue engineering.[18,19] It has been shown that DO induces migration of MSCs from the bone marrow to the site.[20,21]

Although DO is recognised as an effective and safe procedure, there are drawbacks associated with it.[22] The major disadvantage relates to the duration of the bone consolidation phase, when the external fixators need to be kept in place for a prolonged period (12 months on average).[5,17,23] During this period complications can arise including pin site infection and fibrous union; patients and family members may experience physical inconvenience and psychosocial burden due to the retained external fixators, and there is a higher rate of fracture at the docking site.[14,24-26] These limitations hamper any large-scale clinical application of DO. The recommended rate of gradual bone lengthening as described by Ilizarov is 1 mm per day. Higher rates (1.3 mm/day or greater) may lead to tissue damage, and researchers have found that the size of the fibrous zone increased more quickly than that of the new bone zone in these conditions.[27] Also, the bone-regenerating capability slows with increasing age.[28]

To reduce the time required for the DO process, numerous animal models were developed to mimic clinical situations and test the new treatments.[29] MSCs have been used in a variety of applications owing to their regenerative potential and migration capacity. One of the most important capabilities of MSCs is their migration capacity in response to signals released from the injured tissues.[30,31] The application of MSCs has been shown to improve the quality and quantity of bone healing including fractures or DO.[3,32,33]

Materials and Methods

This review included studies that have been published in English; PubMed, OVID and Google Scholar search engines were used. The key terms "cells" and "distraction osteogenesis" were searched, and 434 titles identified. The inclusion criteria were as follows: DO with bone lengthening and administration of MSCs with control and/or comparison group(s). The exclusion criteria included: distraction of other areas such as mandibular distraction; use of bone grafts in addition to DO; testing of new distractor device or new surgical technique; and outcomes assessing soft-tissue changes, rather than bony outcomes. Review articles, case reports and non-English language publications were excluded.

Results

A total of 16 studies meeting the eligibility criteria were selected from 423 published articles. Six studies used small animal models (rats or mice) and six studies used rabbits, while the other three studies used large animal models (two used dogs and one a goat) and one study involved a human subject (Table I). Among these studies, there were variations in the experimental design, including distraction time point, cell transplantation time point, administration methods and cell quantity, as well as outcome assessment methods. All studies used unilateral limb DO with or without cell injection.

The DO protocols are summarised in Table II. Various custom-made or commercially available distractors/lengtheners were used. The latency phase ranged between four and fourteen days. The distraction rate ranged from 0.5 mm/day to 2 mm/day, the total distraction gap ranged from 1.5 mm to 60.8 mm, and the consolidation period ranged from four days to ten weeks.

As shown in Table III, different cell sources were used: autologous or allogenic bone marrow MSCs from long bone[16,20,37,43,45,48,50–52] and iliac crest,[30,38,44,46,47] autologous adipose stem cells,[48,52] and location not specified.[15,49] The number of transplanted cells ranged from 1 to 30×10^6. The cell injection timing included three different time points: in three studies, MSCs were injected during the distraction phase[15,37,49] or during the consolidation phase;[30,38,43,44,45,47,48,51] in three studies, the MSCs were injected on the day of the operation;[16,20,50] and in one study the time of injection was not stated[52]. The majority of the studies reported that MSC transplantation at the end of the lengthening phase with MSCs over 1 x 10^6 enhanced bone consolidation or formation in DO in animal models.

All studies showed a positive effect in bone regeneration on the cell-treated side. This was regardless of whether

Table I. Characteristics of animal models and the main outcomes of the study

First author name and Year	Animal	Gender (n)	Cells Used	Control Group	Main Results	Objectives
Yuji Takamine, 2002[42]	Rat	Male (73)	BMSCs	Collagen gel	Cells treated group was significantly better than that of control group	Promote new bone formation and shorten the consolidation period.
Kazuhiko Kinoshita, 2008[37]	Rabbit	Male (54)	BMSC	Saline	Same as above	Promote bone regeneration of DO
Koichiro Sato, 2010[43]	Rabbit	Male (8)	BMSCs	PBS	Same as above	Promote new bone formation.
Qing-Guo Lai, 2011[44]	Rabbit	Male (54)	BMSCs	Saline	Same as above	Promote bone formation.
Masahito Fujio, 2011[15]	ICR mice	Female (83)	BMECs+SDF-1	Saline	Same as above	Shorten the treatment period of DO.
Jan Gessmann, 2012[45]	Human	Male(6) female(2)	BMSC	N/A	Same as above	Promote bone regeneration of DO
Ozgur Sunay, 2013[46]	Rabbit	Female (21)	BMSCs	Saline	Same as above	Promote new bone formation and shorten bone consolidation phase.
Issei Nomura, 2014[47]	Rat	N/A(60)	ADSC+ Collagen gel	Saline	Same as above	Promote bone regeneration of DO
Yuji Ando, 2014[48]	ICR mice	Female (12)	BMSCs	FBS	Same as above	Shorten the distraction period.
Yohei Harada, 2015[20]	Rabbit	Male (42)	BMSCs + PBS	PBS	Same as above	Repair of large bone defects.
J. J. Zeng, 2016[49]	Dog	Male (27)	BMSCs transfected with hBMP-2	PBS	Same as above	Promote bone regeneration of DO
Xu jia,2016[50]	Rabbit	Male(24)	Human fetal MSC secretome	PBS	Same as above	Promote bone regeneration of DO
Mohammad Mehdi Dehghan, 2015[36]	Dog	Male (10)	MSC+PRP	PRP	Same as above	Promote new bone formation and shortened the consolidation period.
El Hadidi, 2016[29]	Goats	Female (12)	BMSCs	PBS	Same as above	Improve the quality and quantity of DO.
Alexander R. Zheutlin, 2016[16]	Lewis rats	Male (30)	BMSCs	N/A	Same as above	N/A
Sung Joo Lee, 2016[51]	Rabbit	Male (32)	ADSC	Fibrin glue	Same as above	Promote bone regeneration of DO

Table II. Characteristics of distraction osteogenesis protocols

First author, year	Latency time (days)	Rate of lengthening (mm/day)	Total lengthening (mm)	Consolidation phase (days)	Infection rate
Yuji Takamine, 2002[42]	7	0.5	5.0	14/28/42/56	N/A
Kazuhiko Kinoshita, 2008[37]	5	2.0	8.0	N/A	N/A
Koichiro Sato, 2009[43]	7	0.5	10	21	N/A
Qing-Guo Lai, 2010[44]	6	0.8	4.8	42	N/A
Masahito Fujio, 2011[15]	5	0.4	3.2	N/A	N/A
Jan Gessmann, 2012[45]	N/A	N/A	Average 82.4	N/A	Six local pin infections
Ozgur Sunay, 2013[33]	7	0.7	10.5	56	N/A
Issei Nomura, 2014[46]	7	0.8	6.4	14/28/42	N/A
Yuji Ando, 2014[47]	3	0.8	3.2	N/A	N/A
Yohei Harada, 2015[38]	14	N/A	1.5	28/56/84	N/A
J. J. Zeng, 2015[48]	5	1.0	10	14/28/42/56	No infection
Xu Jia, 2015[49]	5	1.0	10	42	N/A
Mohammad Mehdi Dehghan, 2015[36]	7	1.0	60.8	120	N/A
El Hadidi, 2016[29]	5	1.0	10	30	Most animals
Alexander R. Zheutlin, 2016[16]	4	0.6	5.1	28	N/A
Sung Joo Lee, 2016[50]	5	3.0	10	28/56/84	1

the cell sources, cell number, and time points have different effect.

Discussion

In normal DO, the recruitment, proliferation, and osteogenic differentiation of MSCs are sufficient to achieve bone regeneration in the distraction gap (Fig. 1). However, the MSCs may be compromised under conditions such as poor vascularity, severe trauma and radiotherapy. This may result in fibrous union or nonunion.[10,29,34] In order to enhance the quality and quantity, and the time to bone formation in DO, DO animal models were developed to test bone regeneration by MSC therapy approaches.[35]

In all studies reported, the MSC-treated group showed improvement in the quality and quantity of new bone formation in the distraction gap. Dehghan et al,[36] Lee et al,[51] and Kinoshita et al[37] used the higher rate of lengthening, and the MSC therapy significantly promoted new bone formation and shortened the consolidation period in their DO models.

The total size of the distraction gap is another important factor to consider; at present, most studies conducted ⩽ 10 mm of distraction, which did not reflect the real clinical scenario where most DO treatment exceeds 5 cm or more. In the large gap, the effect of cell therapy may be more or less apparent. This needs to be tested in a clinical setting.

Table III. Characteristics of transplanted cells

First Author Name, Year	Cell Type	Cell Source	Cell Number	Time	Passage of MSC
Yuji Takamine, 2002[42]	Allogenic	Femurs	0.1M	When distraction phase finished	3
Kazuhiko Kinoshita, 2008[37]	Autologous	Iliac crest	10M	When distraction phase finished	3
Koichiro Sato, 2010[43]	Allogenic	Iliac bone	30M	When distraction phase finished	3-6
Qing-Guo Lai, 2011[44]	Autologous	Tibia	10M	When distraction phase finished	3
Masahito Fujio, 2011[15]	N/A	N/A	N/A	Every other day from day 4.	3-6
Jan Gessmann, 2012[45]	Autologous	iliac crest	2ml	At the end of the distraction phase	3
Ozgur Sunay, 2013[46]	Autologous	Inguinal regions	5M	When distraction phase finished	3
Issei Nomura, 2014[47]	Autologous	Femurs	1M	after termination of distraction	3
Yuji Ando, 2014[48]	Human MSCs	N/A	0.3M	The second day at the distraction phase	3
Yohei Harada, 2015[20]	Autologous	Tibia	1M	At surgery day	4-6
J. J. Zeng, 2016[49]	Allogenic	Tibia	1M	At surgery day	3
Xu jia,2016[50]	Allogenic	Tibia	N/A	Every 3 days when distraction phase finished	3
Mohammad Mehdi Dehghan, 2015[36]	N/A	Tibia	10M	Middle and end of the distraction phase	3
El Hadidi, 2016[29]	Allogenic	Iliac crest	15M	Day 10 and 20 in the consolidation phase	3
Alexander R. Zheutlin, 2016[16]	Allogenic	Femurs and ummers	2M	At surgery day	3
Sung Joo Lee, 2016[51]	Autologous	Tibia	3M	N/A	3

M, number of cells in millions.

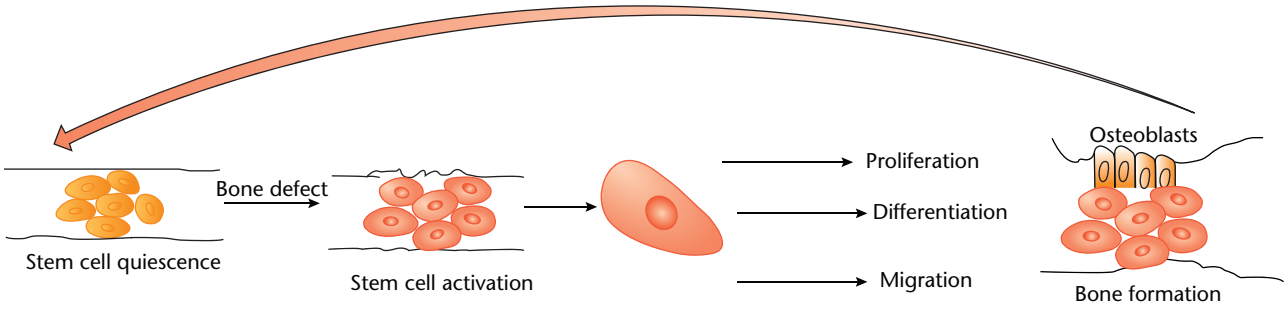

Fig. 1

Summary of studies on the cell therapy including time and number of transplanted cells used.

The timing and number of MSCs for cell injection also varied among studies. MSC isolation and cultivation are similar, though they differ from the source. All of them used MSCs at passages 2 to 6, but only one was characterised by CD33, CD45 and CD90. Most authors did the cell injection at the end of the lengthening period and used a dose of 1 x 10^5 to 3 x 10^7 MSCs, based on previous reported studies (Fig. 2). No clear explanation was given as to why a certain time was chosen for cell delivery. As shown in Tables II and III, the larger the distraction gap, the more stem cells may be needed.

None of the studies has purposely investigated what the optimal cell numbers and optimal time points are for injection. Nor has any study compared the difference between autologous or allogenic MSCs in DO.

The gender of animals may affect the outcome, however, no study has directly compared the effects of cell therapy on the DO process between male and female animals of any species. We know much more about the role of gender in medicine, such as the fact that women are more sensitive to cardiological medicine[38] and women who are lactating may have worsened symptoms. With the increasing demand to eliminate gender bias in animal studies,[39] future efforts are warranted to clarify gender-related differences of cell therapy in DO.

Another issue related to animal models is animal age and skeletal maturity. All animals used were skeletally mature, except the goats used by El Hadidi et al[29] which were of an age comparable with childhood. Older rats display delayed healing of femoral fractures. As DO may be performed on patients of all ages, it is important clinically to interpret the data obtained from animal studies. This is in specific consideration of its application to humans of different skeletal ages. Future studies are needed to compare age-related differences on MSC therapy in DO. Through extensive research, it has become clear that different animal models may have a different optimal DO protocol. Three aspects should be considered carefully during the DO procedure: site of lengthening; lengthening rate; and length.[40,41] The optimal rate of lengthening in small and large animals is commonly thought to be 0.5 mm/day and 1 mm/day, respectively, but there have been attempts using a higher rate of lengthening to create a poor bone formation model.

Adverse events, such as infection, were not carefully or purposefully investigated in any of the 16 studies using

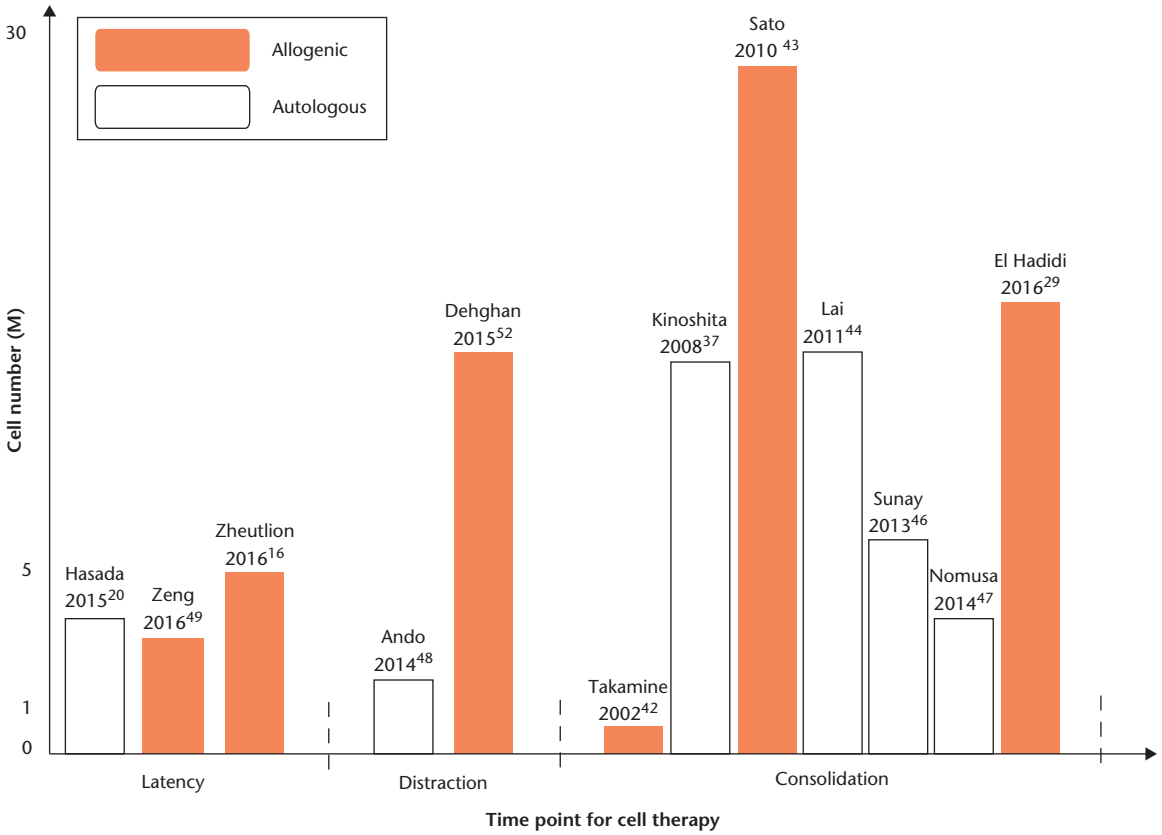

Fig. 2

The role of MSCs in bone defect. MSCs are maintained in quiescence and transiently activated by damage. The use of MSCs to treat damage is attractive as it would implement a reparative process that should be in place and time. MSCs improved the damage healing affecting the callus. Regeneration is followed by two steps, increased migration and proliferation. MSC migration at the defect site is time-and dose-dependent. They must self-renew to produce more stem cells, maintaining tissue homeostasis. MSCs possess the capability to differentiate directly into subsequently bone-forming osteoblasts.

MSC therapy in DO. Most studies did not mention the infection risk assessment, and animals were simply excluded from the study due to pin site infections. Complications related to MSC therapy during limb lengthening procedure must be taken into consideration. In clinical situations, the emphasis is on the use of the GMP facility to process and prepare the cells in order to minimise the potential risks of infection.

Questions related to animal models, DO protocols and cell transplantation regimes still need further investigation. A consensus is required for the development of such a model. Clinical trials using MSC therapy for enhancement of bone formation and consolidation in patients with DO treatment are warranted, and it is the only way to obtain a definitive answer in this subject area.

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Author Contribution

- Y. Yang: First author, Literature review, Manuscript writing.
- S. Lin: Co- first author, Manuscript revision.
- B. Wang: Second author.
- W. Gu: Corresponding author.
- G. Li: Co-corresponding author.

Conflicts of Interest Statement

- None.