SYSTEMATIC REVIEW

Clinical studies investigating the role of mesenchymal stem cells in healing of fracture non-unions: a systematic review

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Abstract

Objective: To identify and evaluate the effectiveness of mesenchymal stem cells (MSCs) in augmenting healing in fracture non-unions.

Methods: A focused literature search was performed on the PubMed/MEDLINE index using the keywords: "non-union", "mesenchymal stem cells", "bone healing", "MSC", "stem cells", and their MeSH terms. The search was reiterated until the 10th of August 2022. Clinical studies were included that assessed the effect of MSCs on fracture non-unions.

Results: Thirteen human clinical trials, studying a total of 318 participants were identified and studied. MSCs with and without biological or synthetic scaffolds were found to be effective in healing of non-unions.

Conclusion: MSCs has been demonstrated to have promising outcomes in the treatment of bone non-union and tissue engineering methods utilizing MSCs may well prove to be valuable in accelerating the process of bone union. However, clinical application of MSCs as a standard method in achieving union in fracture non-unions requires larger clinical trials with a standardised approach to analyzing outcomes.

Keywords: Medline, Mesenchymal, Stem Cells, Tissue Engineering, Fractures

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Introduction

A functionally sound repair of a fractured bone is reliant on a complex interplay of mechanical factors facilitating adequate stability at the fracture site, and an optimum biological milieu, impairment of either one of which resulting in failure of bone healing, or 'non-union'[1]. Although a number of clinical and radiological parameters have been used in literature to define fracture non-union, it is agreed upon that it reflects cessation of the healing process of a fractured bone, and is often associated with significant patient morbidity[2]. It is described by the United States Federal Drug Administration Council as failure to attain fracture union by nine months following the injury, and for which signs of bone healing are not observed for three months. Others, however, have suggested that in the absence of any radiological sign of fracture healing, this time frame for long bones should be revised to six months[3]. Although skeletal tissue demonstrates a remarkable ability to regenerate and remodel, around 5-10% of cases result in fracture non-union[2]. However, the incidence of non-union in literature largely varies and is more commonly seen in the presence of sub-optimal patient factors such as smoking, diabetes and obesity, as well as in non-patient related factors such as high energy mechanism of injury, infections, and open fractures[4,5]. Adequate stability of the non-union along with use of autologous bone graft facilitates union and has been demonstrated to be largely successful[6]. Despite being the standard method for managing fracture non-unions till day, limited supply of biological bone grafts and their associated donor site morbidity and somewhat unpredictable healing potentials have encouraged the search for other strategies[7]. In view of this, tissue engineering techniques to encourage osteogenesis, including the use of biological osteo-competent tissue such as mesenchymal stromal cells (MSCs) are seen to be a promising new avenue for orthopaedic surgeons to explore[8]. Introducing viable MSCs isolated from bone marrow and other tissues (e.g umbilical cord blood, adipose tissue, placenta) into a biological or synthetic scaffold is hypothesised to facilitate good tissue healing in non-united bone, as well as in degenerative diseases. [9-13] MSCs are excellent cellular candidates as they possess the inherent capability of differentiating into a number of mesenchymal tissue lineages, including musculoskeletal tissue. [14,15] The effectiveness of MSCs has been validated by a large number of animal studies and a few clinical reports, and has been put forward as a theoretically promising strategy to improve the management of fracture non-

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unions, including cost effectiveness, earlier recovery, and lesser morbidity. This paper reviews currently available clinical reports to identify the role of stem cells in augmenting healing in fracture non-union.

Methods

Study selection and search stratagem: This study is a systematic literature review reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[16] (Figure 1) A targeted literature search was performed using the PubMed/MEDLINE index using the keywords: "non-union", "mesenchymal stem cells", "bone healing", "MSC", "stem cells", and their MeSH terms in any possible combinations using the logical operators "AND" and "OR". The inclusion criteria encompassed studies that investigated clinical outcomes following intraoperative instillation of stem cells for the treatment of fracture non-unions, human studies, papers written in the English language and full-text articles. Excluded studies included published abstracts, manuscripts written in a language other than English, case reports, reviews, letters to the editor, conference abstracts, technical notes, expert comments, and studies without readily obtainable data or with inadequate information on the type of intervention and the outcomes. Research that was only reliant on outcomes from animal models was likewise omitted. The remaining pertinent literature was screened for inclusion and to identify other studies of interest, with relevant studies proceeding for further evaluation. The search was reiterated until the 10th of August 2022. Data were documented according to a standardized protocol, where objectives and inclusion criteria were specified in detail.

Data extraction: Four of the six investigators, reviewed all of the filtered manuscripts and collected data using a pre-established proforma. Each article's data was compiled into a Microsoft Excel spreadsheet and sorted according to the orthopaedic intervention done and osteo-inductive tissue used. Data including the first author, the year of publication, study design, sample size, patient demographics, the site under consideration, fracture types, the orthopaedic procedure carried out, clinical outcomes and complications, and the type of cellular therapy used were extracted from the reviewed clinical studies. The absence of pain during weight bearing and the formation of bridging calluses in three of the four cortices of the affected bone on plain orthogonal X-rays were used as the clinical and radiological descriptors, respectively, of fracture healing. The Endnote software (Clarivate Analytics, Philadelphia, PA, USA) was utilized to detect duplicate studies. Two different writers independently gathered, analysed, and reviewed all of the data.

Outcome measures: The primary endpoints this study was to identify were 'time-to-union' and 'bone healing' following instillation of MSCs into sites of fracture non-union.

Results

Literature search: The literature search and cross-referencing resulted in 2630 references. Included were 1043 articles after the studies were initially evaluated based on the abstract and title, and once duplicate articles were excluded. A total of 151 studies were excluded after additional scrutiny of the remaining publications as they solely used an in-vitro approach. In all 160 research articles were disregarded as they made use of animal models. Once full-texts were reviewed, 556 researches were eliminated based on the inclusion criteria, and 162 studies were eliminated because they were review articles or published in a language other than English. Eventually, 13 research [17-29] satisfied our eligibility criteria.

As shown in Table-1, the distance travelled for brain tumour surgery was highest in AYA age group compared to paediatric and older adult population patients (p=0.021).

Clinical outcomes of MSCs in fracture non-union: Literature search revealed 13 clinical studies on the role of MSCs in fracture non-union, with a total of 318 patients between the ages of 15 and 86 (mean: 50.2 years), comprising of 166 male subjects (52.2%) and 152 (47.7%) female subjects. The affected anatomic sites included non-unions of the tibia (n=150, 46.8%), ankle (n=86, 27%) femur (n=33, 10.3%), humerus (n=19, 5.9%), radius/ulna (n=16, 5.03%), metacarpal (n=1, 0.31%), metatarsal (n=1, 0.31%), sacrum (n=1, 0.31%) and pelvis (n=1, 0.31%). All of these clinical studies used the iliac crest or spine for retrieving BMA. The researchers used several MSC preparation methods, some of which included concentration[18-21,26-29] and/or culture[17,22-25,27] expansion, some of which used a biological or synthetic scaffold [17-19,22-24,27,29]. Bone union was evaluated using mainly radiographic and the outcomes revealed are promising, with the majority of patients establishing bone union successfully.[17-29]. The primary end points, that is bone healing and time to union, although not measured in a standardized manner, show promising outcomes with 267(83.9%) cases having achieved union at the fracture site, and have been summarized in Table 1.1.

Table-1: Fracture non-unions studied and their outcomes when treated with MSCs

S.NO	AUTHOR	PATIENTS	DIAGNOSIS	HARVESTING METHOD	INTERVENTION	INJECTED VOLUME	OUTCOME
1	Quarto et al [17]	3	Bone defects of tibia, ulna, humerus	BMA then culture expansion into osteoprogenitor cells	Progenitor cell coated on HA scaffold and surgically implanted	unknown	Union in all patients by 2 months
2	Toro et al [18]	6	Non-union of humerus	BMA from iliac crest + cortical/cancellous graft	Bone marrow concentrate injection into fracture site	unknown	Union in all in a mean of 3 months
3	Luca et al [19]	38	Non-union of femur, tibia	BMA from iliac crest and concentration, with demineralized bone matrix and platelet rich fibrin	Bone marrow concentrate injection into fracture site	5 to 6 ml	Union in 30 in a mean of 7 months, persistent nonunion in 8
4	Hernigou et al [20]	64	Non-union of tibia	BMA from iliac crest and concentration	Bone marrow concentrate injection into fracture site	50 ml	53 united with median 12 weeks for radiological union
5	Hernigou et al [21]	86	Non-union of ankle	BMA from iliac crest and concentration	Bone marrow concentrate injection into fracture site	150 ml before concentration.After concentration: unknown	Persistent non-union in 16
6	Marcacci et al [22]	4	Bone defect non-union of tibia, ulna, humerus	BMA from iliac crest and culture expansion 3 weeks	MSC coating over bioceramic HA scaffolds + surgery implantation	20 ml	Union in all patients within 8 months
7	Ismail et al [23]	10	Non-union of femur, tibia, humerus	BMA then culture 4 weeks	MSC + HA granules vs iliac crest bone graft + HA granules	10 ml	All united, but average 3 months quicker in treatment group
8	Wittig et al[24]	3	Non-union of tibia, femur	BMA then cultures on collagen microspheres and PRP added	Collagen sponge inserted into fracture site surgically	Unknown	Union with full functional recovery at 1 year, 2 years, and 3 years
9	Centeno et al[25]	6	Non-union of humerus, sacrum, metatarsal, ischium, tibia	BMA from iliac crest then culture 3-25 days	MSC injection into fracture site	unknown	4 fractures united, 1 lost to follow-up, 1 persistent non-union
10	Flouzat-Lachaniette et al [26]	54	Non-union of tibia	BMA from iliac crest and concentration	MSC injection into fracture site	150 ml before concentration.After concentration: unknown	41 united with mean union at 6 months
11	Giannotti et al[27]	8	Non-union of humerus, radius, ulna	BMA from iliac crest then cultured in autologous serum for 10 to 18 days	Injection in fracture site in a fibrin clot construct	2 ml	All united within 10 months
12	Singh et al[28]	12	Non-union/delayed union of ulna, femur, humerus, metacarpal	BMA from iliac crest	Injection in fracture site	20-40 ml	10 united with mean union at 16 weeks, 2 persistent non-unions
13	Le Thua et al[29]	27	Non-union of ulna, humerus, femur, tibia	BMA + concentrate + added to allogenic bone chips	Bone marrow concentrate + Allograft bone chips vs iliac crest bone graft	8 ml	94.4% union by 5 months (MSC)

Discussion

Patients who have non-union report their lives as being "on hold" for the years that their bone is un-united, which adds significantly to their morbidity. They tend to suffer from longer-lasting pain, physical disabilities, mental health problems, more costly medical care, and a slower return to regular work productivity.[30] Therefore, it is crucial to spot and care for individuals who are likely to experience non-union early on following a fracture. Established non-unions also present considerable issues for clinicians, and many current therapies demand difficult reconstructive surgeries. It would therefore be very helpful to address this problem early on with least invasive and effective treatments.

Fracture non-union: Cellular environment, bone matrix, mechanical stability, and growth factors comprise 'the diamond concept' of fracture healing, the impairment of which is implicated in the etiology of fracture non-union.[31,32] Although no singular definition has been agreed upon to define 'non-union', we consider it to be fractures that do not unite in the anticipated time period and are unlikely to unite without intervention.[32]

Non-union is said to be established by the American Food and Drug Administration when a minimum of nine months has passed or the fracture has not manifested any observable, progressive indications of healing for three months". [33] When evaluating a non-union during clinical examination, a finding of motion and/or pain at the fracture site is indicative of non-union.[34] On the other hand, the lack of bridging callus in at least three of the four cortices is a recognized radiographic criterion that can direct the physician to the diagnosis of a non-union.[35] Since non-union is commonly accompanied by instability at the fracture site, clinical examination is just as critical to diagnosis as the radiological evaluation.

Fracture healing: at the molecular level: A non-union signifies a hinderance in the evolutionary process that encompasses the typical physiological phases which allow a fractured bone to unite. Failure to do so ensues because of factors that upset either its biological or mechanical environment.[5] Notions such as 'the diamond concept' signify that stem cell therapy alone cannot enable fracture union, but instead requires an all-inclusive approach to confront both the mechanical and biological factors involved.[31] These together create the optimum microenvironment required for bone healing. Stem cells play a crucial role in welding these elements together. MSC, being multipotent cells, possess the ability to differentiate into osteoblasts which are osteogenic,[36] and chondroblasts that mature to help form soft callus which serves as a framework with osteo-inductive properties.[36,37] This framework provides a firmness to the fracture site, thus permitting development of hard callus.[37] The function and ability of MSCs to differentiate is directly regulated by a number of growth factors, amongst the most vitals of which are bone morphogenic proteins (BMP).[38]

Stem cell biology: The optimum method to yield and prepare stem cells ought to be inexpensive, and readily available and delivered with little invasiveness. It should also bring forth a consistent and measurable harvest with minimal-to-none loss of cells. Hernigou et al established a linear association between the quantity of injected progenitor cells and the bulk of callus formed at four months. Cases treated with fewer than 30,000 cells were demonstrated to be unsuccessful.[20] On the other hand, patient who developed non-union were seen to have fewer MSC than patients who healed in time and MSC from elderly donors demonstrated decreased proliferative ability, thus reflecting the vitality in understanding the factors affecting the efficacy of MSCs in management of fracture non-union.[39,40] In view of these contributing factors, it is important to mention that MSC require the presence of pro-osteogenic growth factors, such as bone morphogenetic peptides (BMP), platelet-derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1), for osteogenesis.[38] BMP are osteo-inducing proteins that influence MSC differentiation, details of which are outside the purview of this report.

Harvesting of MSCs: Currently, stem cell harvesting for the most part comprises percutaneous aspiration and concentration of marrow from the iliac crest, anterior superior iliac spine or the posterior superior iliac spine. Harvesting can also be done from the sternum, ribs and vertebral bodies; however, the pelvis has been seen to yield larger quantities of stem cells and is thus the more commonly used source.[41]

To aspirate bone marrow from the iliac crest, a large bore needle is advanced from the lateral aspect of the iliac crest in the sagittal plane and directed towards the center of the bone without breaching the medial cortex. The needle is then pulled back and readvanced with a rotational movement, pulling back the needle's plunger each time the needle is advanced to generate negative pressure and aspirate bone marrow.[41]

For concentration of MSCs from bone marrow aspirate (BMA), principles of low-speed centrifugation are generally utilized in operation theatres, producing a concentrate that comprises a variety of haematopoietic

progenitor and stromal cells, including MSCs. On the other hand, stem cells may also be cultured from marrow aspirates in labs, since they tend to cling to culture flasks and form colonies.[20,42,43]

Conclusion

In conclusion, the MSCs have been demonstrated to be effective in the healing of bone nonunion in several human clinical studies. As surgical procedures with bone grafting continue to be the standard treatment for managing fracture nonunion, tissue engineering techniques utilizing MSCs have potential to offer invaluable methods to help hasten the completion of bone union. The usage of MSCs either alone or as MSCs-scaffold composites show promising contribution to improved outcome in patients with fracture non-union. However, clinical application of MSCs as a standard method in achieving bone healing in fracture non-unions requires larger clinical trials with a standardized approach to analyzing outcomes.

Limitations: This systematic review has not been registered with The International Prospective Register of Systematic Reviews (PROSPERO).

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