Accepted Article

Journal of Research and Practice on the Musculoskeletal System JRPMS

Mini Review

Stem cells in fracture healing

Efstathios G. Athanasakis

"Evangelismos" General Hospital of Athens, Athens, Greece

Abstract

Bone fractures are the most common injuries seen in emergency departments worldwide. Fracture healing is a complex physiological process. Existing research has proved that Mesenchymal stem cells (MSCs) assist and augment fracture healing. This review is examining the importance of MSCs in bone healing. A literature search was performed using PubMed, and relevant articles were retrieved.

Keywords: Stem cells, Fracture healing, Nonunion

Introduction

Fracture healing

Bone fractures are the most common injuries seen in emergency departments worldwide. Bone tissue has a remarkable capacity of scar-free repair following a fracture, through fracture healing, a process that recapitulates many aspects of embryonic skeletal development[1,2]. The general pattern of secondary (indirect) bone healing, which is the most common form of fracture healing[3], based on endochondral ossification, consists of haematoma formation, inflammation, angiogenesis, cartilage formation (with subsequent calcification, cartilage removal and then bone formation) and bone remodeling[4]. Primary (direct) healing based on intramembranous ossification, with no periosteal reaction or visible callus formation, doesn't occur commonly in the natural process of bone healing and is common in ORIF.

Stem cells

Stem cells can be defined as undifferentiated cells that can proliferate and have the capacity both to self-renew and differentiate to one or more different cell lineages. Stem cells can be classified according to their source. They can be isolated from fetuses, or adult tissue, but the range of cell types to which they can differentiate varies.

Embryonic stem cells (ESCs) result from the isolation and cultivation of cells from the inner cell mass of the blastocyst, that forms at approximately 5 days after fertilization[5]. They are pluripotent, capable of differentiating into cells derived from all three germ layers[6].

Fetal stem cells can be isolated from fetal blood, bone marrow, liver, and kidney[7]. There is a high concentration of fetal stem cells in cord blood. HSCs isolated from cord blood have a demonstrated use in the treatment of hematopoietic and bone marrow disorders[8-10].

Adult stem cells (somatic stem cells), reside in specific tissues and their main role is thought to be that of maintenance and repair of tissues[11]. Hematopoietic stem cells (HSCs) can self-renew continuously in the marrow and differentiate into the full complement of cell types found in blood. Thus they play the role of premier adult stem cells[12]. However, most orthopaedic research has been focused on mesenchymal stem cells (MSCs). Mesenchymal stem cells(MSCs) are stromal cells that have the ability to self-renew and also exhibit multilineage differentiation. MSCs have been retrieved from a variety of tissues like periosteum, muscle, blood, blood vessels, synovium, and fat[13]. They can be obtained from a patient and cultured in vitro and can thus act as autografts, diminishing the concerns of immunogenicity that exist with other graft sources[14]. The principles of isolation, proliferation, and differentiation describe the general goals of stem cell research and provide the basis of the potential of stem cell use in orthopaedics. The sources of MCs are varied. The ideal source should be easily accessible, harvesting should be noninvasive and cells should be rapidly expandable

The author has no conflict of interest.

Corresponding author: *Efstathios G. Athanasakis, MD, "Evangelismos" General Hospital of Athens, 45-47 Ipsilantou Str., 10676, Athens, Greece*

E-mail: *stathisathanasakis@gmail.com*

Edited by: *Konstantinos Stathopoulos*

Accepted *25 June 2019*

by in vitro culture[15]. Autologous bone marrow contains MSCs that can be directly or percutaneously injected into the injury site. However, it is an invasive procedure and often yields a few cells. Adipose tissue is probably the richest source in human body and may provide an abundant population of MSCs[16]. The techniques that have been described for MSCs' isolation and identification include flow cytometry, per plating, cell transfers, and antibody-coated heads.

Proliferation is the process by which MScs are plated in vitro (on animal products usually) and grown in monolayers[17].

Differentiation is the process by which MSCs under appropriate in vitro or in vivo conditions become more specialized cells e.g. osteoblasts, adipocytes, and chondrocytes. This procedure is induced through hormonal, chemical, and mechanical factors. The multipotency and the extensive proliferative ability of MSCs make them very good candidates for biological cell-based tissue repair approaches like fracture healing[18].

Material and methods

A review of the literature was undertaken, and articles published later than 2000 were included. Searches were performed using PubMed in the English language to identify both original and review articles concerning the use of MSCs in fracture healing. Key words used included stem cells, MSCs, bone marrow, adipose tissue, treatment, fracture healing, bone defect, nonunion, pseudarthrosis, delayed union, long bones. The articles were downloaded directly from publishers or other online resources. We excluded as non-relevant those papers that didn't concern the enhancement of fracture healing through the use of stem cells.

Results

Eighteen articles that met our inclusion and exclusion criteria were incorporated in this review. Stem cells have applications throughout the field of orthopaedics. Especially in fracture healing, they play a very important role. MSC's can be used for the treatment of fractures by direct injection, by systemic infusion, as genetically modified cells or through the combination of scaffolds and growth factors. Direct infusion may be better than a systemic infusion of MSC's because only a few migrate to the site of fracture. Gao J and et al. used bone marrow-derived MSCs from rats that were cultured, labeled with 111 In-oxine and infused to the animals and found that these cells were accumulated to the lungs, while only a small percentage was found in the liver and spleen after iv or intra-artery infusion[19]. Schrepfer and colleagues using mouse MSCs' showed that cell trapping in lungs could be reduced with IV Sodium nitroprusside pretreatment, increasing MSC passage through the lung capillaries, and potentially facilitating cell access to injured organs[20].

Nonunion bone fracture is considered a major health problem. Nonunions are categorized as atrophic, hypertrophic, and oligotrophic. Fayaz, Giannoudis, and his colleagues report that the role of stem cells for successful bone regeneration is critical (diamond concept). Osteogenic cells, osteoconductive scaffolds growth factors and the mechanical environment is the diamond model for treating fracture healing, nonunions and large bone defects[21,22]. Such problems can be treated by bone autografts and allografts that can be combined with MSCs taken from bone marrow aspirates. Autologous bone marrow is a very rich source of MSCs and growth factors and can be injected into the injury site directly[23-25].

Current evidence has shown that cells with the MSC phenotype reside within human skeletal muscles have the capability of in vivo bone formation in combination with bone scaffolds[26]. Moreover, MSCs can be used as a tool to deliver bone morphogenic protein-2 (BMP-2) by in vivo and ex vivo approaches acting as conduits for gene therapy. Lieberman and colleagues implanted into the quadriceps muscle of severe combined immune deficient mice, bone morphogenetic protein-2-producing cells. Thus showed that abundant heterotopic bone formation could be induced and large segmental femoral defects in nude rats could be successfully healed[27]. Musgrave and colleagues showed that In vivo injection of different cell populations into the triceps surae musculature of hind limbs of adult immunodeficient (SCID) mice had. As a result the secretion of bone morphogenetic protein-2 and then bone formation[28].

Scaffolds seeded with MSCs have very often a clinical application in tissue engineering and bone regeneration. They support and promote the osteogenic differentiation of MSCs. Thus enhancing fracture healing Xue D and colleagues showed that the combination of HMGB1 gelatin sponges with MSC sheets promotes fracture healing in rat with tibial fractures that treated surgically. The HMGB1 gelatin sponge scaffolds helped the expansion of mesenchymal stem cells (MSCs) and promoted the osteogenic differentiation of MSCs and MSC sheets[29].

Meeson R and his colleagues showed that the administration of VEGF and the CXCR4 antagonist AMD3100 would increase the availability of MSCs and improve fracture healing. They stabilized with an external fixator a 1.5mm femoral osteotomy in rats. The bone volume was increased in animals treated with VEGF and AMD3100[30].

Another interesting finding of Dong P and his colleagues is that melatonin promotes osteoblastic differentiation of MSCs and fracture healing in rats with femoral fracture via NPY/NPY1R signaling[31].

Xu L and colleagues using an open femur fracture model in rats showed that Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing. Sox11 regulates MSC differentiation and migration, and Sox11-modified MSCs may have clinical implication in acceleration of bone union, which can reduce the delayed healing or nonhealing[32]. Sox11-MSCs or con-MSCs were injected into Sprague-Dawley rats through tail vein injection at 4 days after fracture. The healing was monitored radiologically.

Cheung WH and co-workers with their study showed that application of MSCs and LIPUS improved fracture healing compared with MSCs only or no-treatment control. For their study they used Sprague-Dawley (SD) rats. They created closed fractures on rats' right femur shaft and examined the combined results of exogenous MSCs and LIPUS on fracture healing and observed the dynamic migration of exogenously injected MSCs to fracture site[33].

Kodama A et al. studied femoral fractures with delayed healing in rats using a magnetic field using MSCs expressing luciferase. Using radiology and histology they concluded that callus formation and endochondral ossification were improved[34].

Myers TJ et al. treated mice with tibia fracture with the use if IGF-I and MSCs and found that IGF-I treated mice showed increased callus volume compared with untreated or mice treated with MSCs. However mice treated with MSCs showed twice as large callus than controls[35]. In another study the researchers used a mouse model and roded tibial fractures and by using MSC expressing luciferase they concluded that the migration of stem cells at the fracture side depended on doses. Moreover they studied the callus morphology using micro Ct and histology and concluded the importance of the transplanted MSCs to the healing process[36].

Liebergall M and his colleagues evaluated the efficacy of minimally invasive intervention in distal tibial fracture of 24 patients. They aspirated iliac crest bone marrow and peripheral blood, in order to have both mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) respectively. Then they mixed them with demineralized bone matrix (DBM) and injected radiologically into the fracture site of one group. They showed that the median time to union was 1.5 months earlier in one group without any complications[37].

Conclusion

Promoting fracture healing and bone regeneration is an important challenge for orthopaedic surgeons. The effect of stem cells can be very helpful in our attempt to treat fractures and this field has the potential to be developed in the future.

References

1. Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? Mech Dev 1999;87(1-2):57-66.
2. Vortkamp A, et al. Recapitulation of signals regulating embryonic bone formation during postnatal growth and in fracture repair. Mech Dev 1998;71:65-76.
3. Richard Marsell and Thomas A. Einhorn. The biology of fracture healing. Injury 2011;42(6):551-555.
4. Phillips, Overview of the fracture healing cascade. A M Injury 2005;36(3):S5-S7.
5. Tandis Vazin and William J. Freed Human embryonic stem cells: Derivation, culture, and differentiation: A review Restor Neurol Neurosci 2010;28(4):589-603.
6. Pedro C Chagastelles and Nance B Nardi. Biology of stem cells: an overview. Kidney Int Suppl 2011;1(3):63-67.
7. O'Donoghue K, Fisk NM. Fetal stem cells. Best Pract Res Clin Obstet Gynaecol 2004;18(6):853-75.
8. Marcus AJ, WoodburyJ Fetal stem cells from extra-embryonic tissues: do not discard. Cell Mol Med 2008;12(3):730-42.
9. Gepstein L Circ. Derivation and potential applications of human embryonic stem cells. Circ Res 2002;91(10):866-76.
10. Sullivan Banking on cord blood stem cells M J Nat Rev Cancer 2008; 8(7):555-63.
11. Regeneration Stefania Montagnani, Maria A. Rueger, Toru Hosoda, and Daria Nurzynska. Adult Stem Cells in Tissue Maintenance and Stem Cells Int 2016;2016:7362879.
12. Stem Cells and the Future of Regenerative Medicine National Research Council (US) and Institute of Medicine (US) Committee on the Biological and Biomedical Applications of Stem Cell Research Washington (DC): National Academies Press (US), 2002.
13. Woei-Cherng Shyu and Shinn-Zong Lin. Cell Mesenchymal Stem Cells Dah-Ching Ding, Transplantation, Vol. 20, pp. 5-14, 2011.
14. Imran Ullah, Raghavendra Baregundi Subbarao and Gyu Jin. Human mesenchymal stem cells - current trends and future prospective. Rho Biosci Rep 2015.
15. Wang X, Wang Y, Gou W, Lu Q, Peng J, Lu S. Role of mesenchymal stem cells in bone regeneration and fracture repair: a review. Int Orthop 2013;37(12):2491-8.
16. Toosi S, Behravan N, Behravan JJ Nonunion fractures, mesenchymal stem cells and bone tissue engineering. Biomed Mater Res A 2018; 106(9).
17. Fernando A. Fierro, Stefanos Kalomoiris, Claus S. Sondergaard, and Jan A. Nolta Effects on Proliferation and Differentiation of Multipotent Bone Marrow Stromal Cells Engineered to Express Growth Factors for Combined Cell and Gene Therapy Stem Cells 2011;29(11):1727-1737.
18. Augello A, De Bari C. The regulation of differentiation in mesenchymal stem cells. Hum Gene Ther 2010;21(10).
19. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organ 2001;169(1):12-20.
20. Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. Transplant Proc 2007;39(2):573-6.
21. Fayaz HC, Giannoudis PV, Vrahas MS, Smith RM, Moran C, Pape HC, Krettek C, Jupiter The role of stem cells in fracture healing and nonunion. J B Int Orthop 2011.
22. Giannoudis PV, Einhorn TA, Fracture healing: the diamond concept. Marsh D Injury 2007;38.
23. Ateschrang, Ochs BG, Lüdemann M, Weise K, Albrecht D. Fibula and tibia fusion with cancellous allograft vitalised with autologous bone marrow: first results for infected tibial non-union. Arch Orthop Trauma Surg 2009.
24. Goulet JA1, Senunas LE, DeSilva GL, Greenfield ML Autogenous iliac crest bone graft. Complications and functional assessment.. ClinOrthopRelat Res. 1997 Jun;(339):76-81
25. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005;87(7):1430-7.
26. Owston H, Giannoudis PV, Jones E. Do skeletal muscle MSCs in humans contribute to bone repair? A systematic review. Injury 2016; 47Suppl 6:S3-S15.
27. Lieberman JR, Le LQ, Wu L, Finerman GA, Berk A, Witte ON, Stevenson SJ. Regional gene therapy with a BMP-2-producing

murine stromal cell line induces heterotopic and orthotopic bone formation in rodents. Orthop Res 1998;16(3):330-9.
28. Musgrave DS, Bosch P, Lee JY, Pelinkovic D, Ghivizzani SC, Whalen J, Niyibizi C, Huard J. Ex vivo gene therapy to produce bone using different cell types. Clin Orthop Relat Res 2000;(378):290-305.
29. Xue D, Zhang W, Chen E, Gao X, Liu L, Ye C, Tan Y, Pan Z, Li H. Local delivery of HMGB1 in gelatin sponge scaffolds combined with mesenchymal stem cell sheets to accelerate fracture healing. Oncotarget 2017;8(26):42098-42115.
30. Meeson R, Sanghani-Keri A, Coathup M, Blunn G. VEGF with AMD3100 endogenously mobilizes mesenchymal stem cells and improves fracture healing. J Orthop Res 2018.
31. Dong P, Gu X, Zhu G, Li M, Ma B, Zi Y. Melatonin Induces Osteoblastic Differentiation of Mesenchymal Stem Cells and Promotes Fracture Healing in a Rat Model of Femoral Fracture via Neuropeptide Y/Neuropeptide Y Receptor Y1 Signaling. Pharmacology 2018;102(5-6):272-280.
32. Xu L, Huang S, Hou Y, Liu Y, Ni M, Meng F, Wang K, Rui Y, Jiang X, Li G. Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing: Sox11 regulates differentiation and migration of MSCs. FASEB J 2015;29(4):1143-52.
33. Cheung WH, Chin WC, Wei FY, Li G, Leung KS. Applications of exogenous mesenchymal stem cells and low intensity pulsed ultrasound enhance fracture healing in rat model. Ultrasound Med Biol 2013;39(1):117-25.
34. Kodama A, Kamei N, Kamei G, Kongcharoensombat W, Ohkawa S, Nakabayashi A, Ochi M. In vivo bioluminescence imaging of transplanted bone marrow mesenchymal stromal cells using a magnetic delivery system in a rat fracture model. J Bone Joint Surg Br 2012;94(7):998-1006.
35. Myers TJ, Yan Y, Granero-Molto F, Weis JA, Longobardi L, Li T, Li Y, Contaldo C, Ozkan H, Spagnoli A. Systemically delivered insulin-like growth factor-I enhances mesenchymal stem cell-dependent fracture healing. Growth Factors 2012;30(4):230-41.
36. Granero-Moltó F, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, Longobardi L, Jansen ED, Mortlock DP, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells 2009;27(8):1887-98.
37. Liebergall M, Schroeder J, Mosheiff R, Gazit Z, Yoram Z, Rasooly L, Daskal A, Khoury A, Weil Y, Beyth S. Stem cell-based therapy for prevention of delayed fracture union: a randomized and prospective preliminary study. Mol Ther 2013;21(8):1631-8.