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The Role of Osteochondral Progenitor Cells in Fracture Repair
[Association Of Bone And Joint Surgeons Workshop: Fracture Healing Enhancement: Section Ii: Fracture Repair Process]

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Outline

Graphics

Abstract

The repair of a fracture necessarily entails synthesis of osseous tissue requiring the transformation of undifferentiated osteochondral progenitor cells to mature osteoblasts and chondrocytes. Owen and Friedenstein proposed that there are stem cells for all mesenchymal tissues, resident in bone marrow throughout life, that have a lineage comparable to that described for hematopoiesis. Subsequent with this initial study, marrow derived and periosteal derived progenitor cells have been shown to produce bone and cartilage in numerous in vivo and in vitro studies. The differentiation process appears to depend heavily on the influences of numerous cytokines, especially the transforming growth factor beta superfamily. Initial cartilage formation from progenitor cells is important in any secondary fracture repair. In the vitro study of chondrogenesis, the marrow derived progenitor cells were shown to differentiate into their terminal phenotype, the hypertrophic chondrocyte, as indicated by the detection of Type X

collagen messenger ribonucleic acid and protein. A concomitant elevation in the alkaline phosphatase level suggests that these cells are ready to mineralize. Despite the importance of these cells in fracture repair, the characterization of these cells and the mechanism of their differentiation have only begun to be explored.

The repair and regeneration of any tissue requires cell proliferation and new matrix production to bridge the undamaged tissue segments. The cells that effect the repair can be fully differentiated and present locally. Osteoblasts may bridge a very short segmental defect effectively as in a nondisplaced stable fracture. However, the repair of most fractures and the regeneration of a large bone segment requires not only osteoblasts, but a complex interaction between chondrocytes, osteoblasts, and endothelial cells. Although preexisting osteoblasts may be involved to a limited extent, the differentiation of osteochondral progenitor cells into osteoblasts and chondrocytes is the first step in the formation on bone and cartilage matrix in fracture callus. Any failure in the mobilization, proliferation, and differentiation of these progenitor cells will lead to failure in the formation of new bone and to eventual nonunion.

The characterization of these progenitor cells and the cytokines that affect their proliferation and differentiation is essential for understanding the mechanism of fracture repair, and for developing new treatments for clinical problems such as fracture nonunions.20 This review examines the evidence for the presence of these progenitor cells in tissues and the roles they play in the repair of fractured bone.

The healing of a fractured bone is a complex biologic event leading to the restoration of the whole bone itself. In most other tissues, scarlike repair mediated by fibroblasts takes place. However such a response would be wholly inadequate in bone repair. The repair of a fracture necessarily requires synthesis of osseous tissue. This process involves intramembranous and endochondral bone formation, requiring the transformation of undifferentiated osteochondral progenitor cells to mature osteoblasts and chondrocytes.10,45,69 These cells of the mature phenotype eventually fill the fracture gap with newly synthesized bone and cartilage forming a fracture callus.

Clinically, a successful fracture repair requires formation of new bone matrix bridging the cleavage gap between the damaged ends of the bone and having the necessary strength to withstand load applied to it. This process requires the mobilization, replication, and differentiation of locally present osteochondral progenitor cells responding to the local and systemic effects of injury.10

The importance of the cellular response to the repair process has been well accepted, but the sources of the progenitor cells still are debated. Although the early authors disagreed as to the relative importance of the periosteum and bone marrow as sources of progenitor cells for fracture repair, for more than a century it has been theorized that both tissues are involved. In the mid1800s, Dupuytren 19 proposed that the cartilage of fracture callus originated from periosteum and bone marrow. There is now good evidence to suggest that bone marrow and the

inner cambium layer of the periosteum contain progenitor cells and that both are involved in fracture repair.45

Although it is intuitive that osteochondral progenitor cells are present in association with bone, this may be a limited view of progenitor cells. There is evidence to suggest that osteochondral progenitor cells are present not only in association with osseous tissue, but also throughout the various nonosseous tissues. This presence of the progenitor cells in muscular tissue is seen by the formation of heterotopic ossification in sites such as the quadriceps femoris after a soft tissue trauma.

The recent use of demineralized bone matrix and BMP2 induction of osteogenesis in animal models of posterolateral spine fusion also show that there must be osteoprogenitor cells present in the heterotopic sites such as the posterior musculature and the other soft tissues surrounding the vertebral column.9,21,30,42,50,58,59,63,67 These progenitor cells in muscles also may play a role in providing cells to the initial fracture callus. Others theorize that perivascular mesenchymal cells or even circulating mesenchymal cells are important for fracture repair. At present, the exact source of progenitor cells destined to differentiate into the osteoblasts and chondrocytes of fracture callus is not known, although periosteal and marrow cells most likely contribute to the repair process.10,45

Despite the acknowledged importance of these cells in fracture repair, their manipulation and use to achieve bone regeneration and repair have not been studied extensively. Furthermore, the characterization of these cells and the mechanism of their differentiation have only begun to be explored.

In primary bone repair, bone to bone contact and rigid fixation allow union of bone with a paucity of fracture callus during the repair phase. In this process, the need to mobilize progenitor cells for the repair may not be great because the fracture gap is nominal. However, even in this ideal condition, excessive stripping of the periosteum may lead to nonunion by depriving the fracture repair site of periosteal osteochondral progenitor cells and a blood supply.

In circumstances wherein a fracture is not rigidly fixed, secondary bone repair ensues, mediated by the formation of a large fracture callus. The number of cells contributing to the fracture callus by the marrow or periosteum may vary according to the location of the fracture and the amount of periosteal damage present. Bone formation in the callus includes initial intramembranous bone formation mediated by the cells undergoing primary osteoblastic differentiation and secondary endochondral bone formation mediated by preceding chondrocytic differentiation of progenitor cells and eventual secondary ossification of the chondroid matrix 45 (Fig 1). In the metaphyseal areas of bone, where a large amount of the cancellous bone is present, nonunions rarely develop because because of an extensive vascular supply and the presence of abundant marrow progenitor cells at the fracture site. Therefore, fractures in these areas heal readily, in contrast to the slowly healing diaphyseal fractures.

Fig 1. Photomicrograph of healing bone showing abundant cartilage formation between the two bone ends (toluidine blue staining ×40).

One of the most clinically difficult situations in fracture treatment involves a large segmental loss of bone. When a critically large fracture gap exists, there may not be enough fracture callus to bridge the two ends of the fractured bone sufficiently. In these difficult circumstances, various strategies have been used to bridge the large bone segment defects, all with some success, but usually at a tremendous cost in time and effort to the patient and physician.

The most frequently used and well accepted strategies for fixing segmental defects involve traditional bone grafting techniques using either fragments of autologous bone packed into the defect or a block of structural bone transferred to bridge between the fractured ends of the bone. In these situations the structural bone matrix of the transplanted bone and the bone marrow containing osteoprogenitor cells are delivered to the fractured site.

The creation of bone in large fracture gaps is, in many ways, no different than the creation of an intertransverse process fusion mass in the posterior spinal arthrodesis by the traditional morselized bone graft technique, or the creation of an anterior interbody fusion after discectomy by using a transported block graft such as a tricortical iliac crest graft. In autologous bone grafting of fractures, numerous morselized bone fragments or a single structural bone graft is placed in contact with the bones or bone ends to be united. The undifferentiated progenitor cells from the marrow of the autologous bone graft and the marrow and periosteum of the local bone are stimulated by the fracture environment to differentiate into osteoblasts or chondrocytes, forming a bridging osteochondral tissue.

chondrocytic appearance of cells, and the immunochemical detection of Type II collagen show clearly that the tissue generated by these marrow derived cells is cartilage (Fig 3).

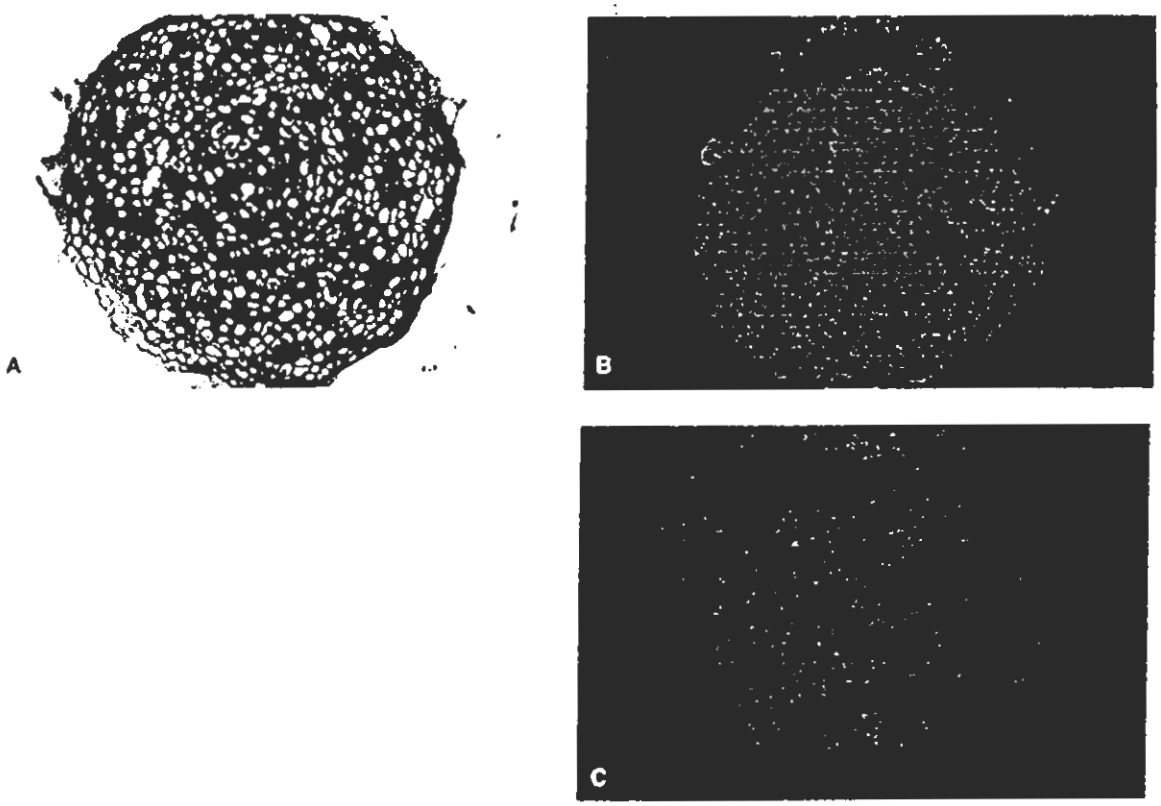

Fig 3A-C. Frozen sections of rabbit bone marrow derived osteochondral progenitor cells 3 weeks after in vitro aggregate culture. (A) Toluidine blue staining. (B) Antitype II collagen immunohistochemical staining. (C) Antitype X collagen immunohistochemistry. Photographed at ×20 magnification.

In this cell aggregate culture system, not only do these marrow derived progenitor cells differentiate into chondrocytes, but the cells differentiate additionally into their terminal phenotype, the hypertrophic chondrocyte, as indicated by the detection of Type X collagen mRNA and protein. The appearance of Type II and Type X collagen mRNA and the disappearance of Type I collagen mRNA by 7 days after aggregation indicate a change in the phenotypic expression of these cells.

The synthesis and deposition of Type X collagen are phenomena that occur rapidly after the appearance of Type II collagen.[34] As stated earlier, this transformation of progenitor cells to chondrocytes is important in fracture repair because much of the secondary repair is mediated by early cartilage formation at the fracture site, hypertrophy of the chondrocytes, mineralization of the cartilage, and eventual secondary ossification.[10,45,69] The early appearance of Type II collagen and the rapid subsequent appearance of Type X collagen also are seen in fracture callus.[3,32,62,67] The similarity between the in vitro aggregate culture system of marrow derived

On the basis of their work, Owen and Friedenstein 51 defined the marrow stromal cell lineage from stem cell to fibroblasts, adipocytes, osteogenic cells (possessing osteogenic and chondrogenic potential) and reticular cells (hematopoietic support cells).51 More recently, Caplan 11 proposed a slightly refined version of this system, the mesengenic process, whereby the mesenchymal stem cell can enter the bone, cartilage, tendon, muscle, ligament, adipocytic, or hemopoietic support cell lineages. As Caplan saw it, development and aging can be viewed as a continuum, with the age related loss of mesenchymal tissue regenerative capacity attributable to the decrease in number of mesenchymal stem cells. Regardless of the definition used, there is still no proof of the stem cell's existence, but the hypothesis is attractive, spurring scientists to explore marrow mesenchymal cell differentiation.

After the isolation and expansion of marrow derived culture plate adherent cells, Friedenstein et al 25 implanted the cells in vivo, contained in diffusion chambers, and observed bone formation. Since then, numerous studies have been done to extend the data on the in vivo osteogenic and chondrogenic capacity of these cells isolated from animal and human marrow.4,5,7,24,27,33 Isolated periosteal derived cells also have been shown to possess this potential on reimplantation in vivo 46,47,64 (Fig 2).

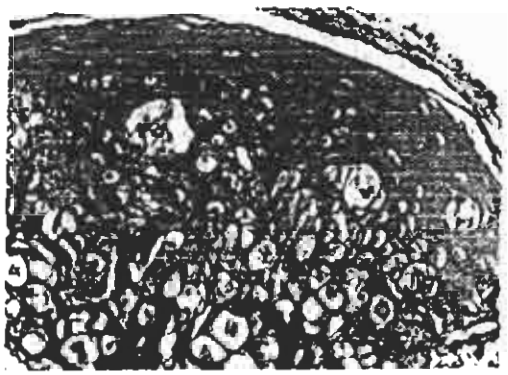

Fig 2. Photomicrograph showing bone and cartilage formation in a collagen sponge loaded with rabbit bone marrow derived osteochondral progenitor cells harvested 3 weeks after subcutaneous implantation in vivo in an athymic mouse (toluidine blue staining ×40).

In addition to the in vivo studies of isolated progenitor cells, their differentiation has been explored in vitro with osteogenesis and chondrogenesis assays. The formation of osseous tissue, in the form of bone nodules, is a well recognized assay for the osteogenic potential of these cells.8,43 As stated earlier, the differentiation of progenitor cells to osteoblasts and chondrocytes occurs during fracture healing. The maturation of the cartilaginous fracture callus is analogous to growth plate chondrocyte maturation and secondary bone formation. Thus, for the scientific study of fracture repair, it is of interest that along with the success of producing osteogenesis in vitro, chondrogenesis also has been achieved in vitro with avian and embryonic mammalian progenitor cells and cell lines.1,2,12,17,28

Recently, the authors have described the chondrogenic differentiation of bone marrow derived progenitor cells obtained from pluripotential cells of postnatal mammalian bone marrow using an in vitro cell aggregate culture system.34 The presence of a metachromatic staining matrix, the

In animal experiments, the use of bone marrow in combination with autologous bone, heterologous bone, extracted demineralized bone matrix, and osteoinductive carriers such as ceramics has been explored. Using a rabbit spinous process fusion model, Lindholm et al 40,41 found that a combination of demineralized bone matrix with bone marrow cell aspirates gave a faster and more stable fusion than did autogenous bone or either component alone. Curylo et al 16 also reported improvement in the rate of spinal arthrodesis and a greater quantity of bone formed when bone marrow was added to autogenous bone graft in a rabbit posterolateral spine fusion model. In an orthotopic bone site, Grundel et al 29 described the effective union of a rabbit ulna gap model using a combination of marrow aspirate and biphasic calcium phosphate ceramic. A likely reason for the beneficial effect of the bone marrow in these studies was the presence of the osteoprogenitor cells in the marrow aspirates.

Numerous studies suggest that a carrier such as demineralized bone matrix, bone, or hydroxyapatite may not be needed when bone marrow is used to augment healing in an orthotopic site such as that of a femoral shaft defect. Takagi and Urist 61 used bone morphogenetic protein and bone marrow aspirate to treat large femoral gap defects in rats and reported high rates of union. Paley et al 52 and Grundel et al 29 described the beneficial effect of percutaneous bone marrow grafting of bone defect in animal models. They showed that the successful union can be achieved in an orthotopic site without any growth factors or carriers. Werntz et al 66 effected biologically stable regeneration of the osseous defect and successful union of the bone with the addition of bone marrow to an experimental rat femoral shaft defect model. With a sufficient amount of marrow, the rate of fusion and the biomechanical strength of the repair were similar to those of autologous bone graft repair. However, injection of dead marrow, with cells lysed by repeat freeze thawing, did not effect significant bony repair, showing that progenitor cells in the marrow are important in promoting bone formation.66

The effectiveness of bone marrow in fracture repair has been shown clinically. In the treatment of tibial nonunion, Connolly et al 14 injected autologous marrow percutaneously to the fracture site and observed healing of the nonunion in 80% of the patients immobilized in casts and 100% of the patients with intramedullary rod fixation. It was concluded that bone marrow injection was as effective in the treatment of tibial nonunion as autologous bone grafting.

Bone marrow and periosteum have the capacity to induce bone formation in orthotopic and heterotopic sites.18,27,31,40,46,47,68 Thus, the isolation, characterization, and use of the progenitor cells that produce this phenomenon are important research topics. Owen and Friedenstein 51 proposed that stem cells for all mesenchymal tissues are resident in bone marrow throughout life, and that they have a lineage comparable with that described for hematopoiesis. These authors established that cells isolated from postnatal bone marrow have the capacity to differentiate into bone, cartilage, and adipocytes. Because this work was done with cell populations that were not cloned, there is no proof that all the phenotypes found came from the same stem cells.51 There must be mesenchymal progenitor cells in the bone marrow population, but whether true stem cells are present is not yet proved. If present, they are thought to constitute a minor proportion of the heterogenous marrow population, and to contain many mesenchymal progenitor cells at different stages of differentiation, a situation analogous to that found in cells of the hematopoietic system.

At times, the amount of bone required to bridge the gap exceeds the autologous cancellous bone available for grafting. These cases are treated by a free vascularized bone transfer or by distraction osteogenesis. However, the surgery for vascularized fibula implantation requires a specialized physician with expertise and knowledge concerning microvascular surgery. The treatment is labor intensive and amenable to a only few specialists. Local bone transport by distraction osteogenesis is an effective method for filling segmental defects or lengthening bone. This local bone transport technique involves corticotomy with progressive distraction of the fracture callus as in the use of the Ilizarov external fixator. Its usefulness is limited by the reality that the callus can be distracted only at a very slow rate, requiring many months before enough bone is created to bridge the gap. Furthermore, this process frequently requires a second surgery to facilitate complete healing (addition of the autologous bone graft at the docking site of the transported bone to complete the bone to bone union).

Recently, new potential treatment options for the repair of large segment defects have been studied. The use of bioactive factors at the fracture site to stimulate locally present progenitor cells and the delivery of osteoprogenitor cells directly to the fracture site in a greater number than would be present ordinarily are two potentially useful techniques. The early recognized benefit of using demineralized bone matrix was followed by the realization that the benefit probably is related to the osteogenic factors present in the matrix that stimulate differentiation of osteogenic precursor cells.55 These osteogenic factors include the bone morphogenetic proteins. Although it now is recognized that these proteins have roles in the development of many tissues other than bone, it is established that they are capable of inducing osteogenesis by promoting differentiation of progenitor cells. Thus, a strategy using bone morphogenetic proteins to facilitate bone formation has appeared in the literature, and its use has been impressive in orthotopic 15,26,44,60,61,68 and heterotopic sites.9,42,57-59

Although bone morphogenetic proteins alone show great promise, clinical evidence of their usefulness has not yet been reported. Furthermore, the in vivo studies of bone morphogenetic proteins in bone formation generally use young animals. Theoretically, bone morphogenetic proteins may work well on these younger animals because of the abundance of the undifferentiated progenitor cells in the orthotopic or heterotopic bed. However, in the clinical scenario involving older patients, it is not known if there exist sufficient undifferentiated cells capable of responding to these cytokines. Evidence from work on bone marrow shows that there is an age related decrease in these progenitor cells.54 Recently, Fleet et al 23 reported that there is an age related decline in the osteogenic response to BMP2 laden implants in rats. Getting a comparable response in 1 month and 16 month old rats required 12 times the dose in the latter animals, suggesting a deficiency either in number or responsiveness of progenitor cells to the osteogenic cytokines.23

Another new potential treatment option to improve segmental gap healing involves the delivery of the osteoprogenitor cells to the fracture gap. The importance of providing additional osteoprogenitor cells to the site of bone formation has been explored by numerous authors. Many of the studies involved the introduction of bone marrow aspirate into orthotopic and heterotopic sites of bone formation.16,29,40,41,61,66 Although the undifferentiated osteochondral progenitor cells may be found in tissues throughout the body, bone marrow most commonly is used as the source of these cells because of its ready availability and almost unlimited reserves.

progenitor cells and the experimental fracture callus suggest that marrow progenitor cells are involved in fracture repair.

When aggregated progenitor cells undergo successful chondrogenesis, a concomitant elevation in the alkaline phosphatase level is seen, suggesting that these cells are ready to mineralize.34 This rise in alkaline phosphatase activity adds to the evidence that these cells differentiate into hypertrophic chondrocytes. This relatively quick appearance of Type X collagen and alkaline phosphatase activity indicate early mineralization of the cartilage, an important factor for fracture repair.

The induction of chondrogenesis in these cells requires particular culture conditions. The cells were maintained in a format resembling that of the precartilage condensation seen in fetal development.22 In a recent article, Noble et al 48 described experiments in which porcine bone marrow cells grown to confluence on tissue culture plates retracted into nodular structures in which Type II collagen was immunolocalized after 6 days in culture. Thus, as found in the current study, chondrogenesis was induced after the cells formed precartilage condensationlike structures. The formation of cellular condensations in the transformation of progenitor cells to chondrocytes may be important not only in vitro, but also in vivo. Bleeding from the marrow with accumulation at the fracture site may play an important role in the delivery of cells to the fracture. It also may allow the cell to cell interaction so important in the rapid chondrogenic differentiation of those cells forming initial fracture callus.

The importance of osteochondral progenitor cells in fracture repair is evident. Differentiation of these cells into osteoblasts and chondrocytes is the first step in the formation of bone and cartilage matrix in fracture callus. Numerous cytokines appear to be active in promoting differentiation of these progenitor cells. However, specific effects and interactions in normal fracture healing are yet to be clarified.6,13,35-39,49,52,53 Nevertheless, any failure in this differentiation will result in failure to form new bone and eventual nonunion. Additional characterization of these progenitor cells and the cytokines that affect their proliferation and differentiation is essential to understanding the mechanism of fracture repair, and to the development of new treatments for clinical problems such as fracture nonunions.20

References

1. Ahrens PB, Solursh M, Reiters R: Stage-related capacity for limb chondrogenesis in cell culture. Dev Biol 60:69-82, 1977. Bibliographic Links [Context Link]

2. Asahina I, Sampath TK, Nishimura I, Hauschka PV: Human osteogenic protein-1 induces both chondroblastic and osteoblastic differentiation of osteoprogenitor cells derived from newborn rat calvaria. J Cell Biol 123:921-933, 1993. Bibliographic Links [Context Link]

3. Ashhurst DE: Collagens synthesized by healing fractures. Clin Orthop 255:273-283, 1989. [Context Link]

4. Ashhurst DE, Ashton BA, Owen ME: The collagens and glycosaminoglycans of the extracellular matrices secreted by bone marrow stromal cells cultured in vivo in diffusion chambers. J Orthop Res 8:741-749, 1990. Bibliographic Links [Context Link]

5. Ashton BA, Allen TD, Howlett CR, et al: Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. Clin Orthop 151:294-307, 1980. [Context Link]

6. Aspenberg P, Thorngren KG, Lohmander LS: Dose-dependent stimulation of bone induction by basic fibroblast growth factor in rats. Acta Orthop Scand 62:481-484, 1991. Bibliographic Links [Context Link]

7. Bab I, Passi-Even L, Gazit D, et al: Osteogenesis in in vivo diffusion chamber cultures of human marrow cells. Bone Miner 4:373-386, 1988. Bibliographic Links [Context Link]

8. Beresford JN: Osteogenic stem cells and the stromal system of bone and marrow. Clin Orthop 240:270-280, 1989. [Context Link]

9. Boden SD, Schimandle JH, Hutton WC, Chen MI: The use of an osteoinductive growth factor for lumbar spinal fusion. Spine 20:2626-2644, 1995. Bibliographic Links [Context Link]

10. Bolander ME: Regulation of fracture repair by growth factors. Proc Soc Exp Biol Med 200:165-170, 1992. Bibliographic Links [Context Link]

11. Caplan AI: Mesenchymal stem cells. J Orthop Res 9:641-650, 1991. Bibliographic Links [Context Link]

12. Caplan AI, Stoolmiller AC: Control of chondrogenic expression in mesodermal cells of embryonic chick limb. Proc Natl Acad Sci USA 70:1713-1717, 1973. Bibliographic Links [Context Link]

13. Cheng SL, Yang JW, Rifas L, et al: Differentiation of human bone marrow osteogenic stromal cells in vitro: Induction of the osteoblast phenotype by dexamethasone. Endocrinology 134:277-286, 1994. Bibliographic Links [Context Link]

14. Connolly JF, Guse R, Tiedemán J, Dehne R: Autologous marrow injection as a substitute for operative grafting of tibial nonunion. Clin Orthop 266:259-270, 1991. Bibliographic Links [Context Link]

15. Cook SD, Baffes GC, Wolfe MW, et al: The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. J Bone Joint Surg 76A:827-838, 1994. [Context Link]

16. Curylo LJ, Johnstone B, Petersilge S, et al: Autogenous bone marrow augmentation of spinal fusion in a rabbit posterolateral fusion model. Trans Orthop Res Soc 22:356, 1997. [Context Link]

17. Denker ARE, Nicoll SB, Tuan RS: Formation of cartilage-like spheroids by micromass cultures of marine C3H10T1/2 cells upon treatment with transforming growth factor-beta 1. Differentiation 59:25-34, 1995. Bibliographic Links [Context Link]

18. Dennis JE, Haynesworth SE, Young RG, Caplan AI: Osteogenesis in marrow-derived mesenchymal cell porous ceramic composites transplanted subcutaneously: Effect of fibronectin and laminin on cell retention and rate of osteogenic expression. Cell Transplant 1:23-32, 1992. Bibliographic Links [Context Link]

19. Dupuytren C: On the Injuries and Diseases of Bones: Being Selections from the Collected Edition of the Clinical Lectures of Baron Dupuytren. In Le Gros Clark F (ed and translator). London, Sydenham Society 1847. [Context Link]

20. Einhorn TA, Majeska RJ, Rush EB, et al: The expression of cytokine activity by fracture callus. J Bone Miner Res 10:1272-1281, 1995. Bibliographic Links [Context Link]

21. Feiertag MA, Boden SD, Schimandle JH, Norman JT: A rabbit model for nonunion of lumbar intertransverse process spine arthrodesis. Spine 21:27-31, 1996. Ovid Full Text Bibliographic Links [Context Link]

22. Fell HB: The histogenesis of cartilage and bone in the long bones of the embryonic fowl. J Morphol Physiol 40, 417-459, 1925. [Context Link]

23. Fleet JC, Cashman K, Cox K, Rosen V: The effects of aging on the bone inductive activity of recombinant human bone morphogenetic protein-2. Endocrinology 137:4605-4610, 1997. Bibliographic Links [Context Link]

24. Friedenstein AJ: Precursor cells of mechanocytes. Int Rev Cytol 47:327-355, 1976. Bibliographic Links [Context Link]

25. Friedenstein AJ, Deriglasova UF, Kulagina NN, et al: The development of fibroblast colonies in monolayer cultures of guinea pig bone marrow and spleen cells. Cell Tissue Kinet 3:393-403, 1970. Bibliographic Links [Context Link]

26. Gerhart TN, Kirker-head C, Kriz MJ, et al: Healing segmental femoral defects in sheep using recombinant human bone morphogenetic protein. Clin Orthop 293:317-326, 1993. [Context Link]

27. Goshima J, Goldberg VM, Caplan AI: The osteogenic potential of culture-expanded rat marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. Clin Orthop 262:298-311, 1991. [Context Link]

28. Grigoriadis ARE, Heersche JNM, Aubin JE: Continuously growing bipotential and monopotential myogenic, adipogenic, and chondrogenic subclones isolated from the multipotential RCJ31 clonal cell line. Dev Biol 142:313-318, 1990. Bibliographic Links [Context Link]

29. Grundel RE, Chapman MW, Yee T, Moore DC: Autogeneic bone marrow and porous biphasic calcium phosphate ceramic for segmental bone defects in the canine ulna. Clin Orthop 266:244-258, 1991. [Context Link]

30. Guizzardi S, Di Silvestre M, Scandroglio R, et al: Implants of heterologous demineralized bone matrix for induction of posterior spinal fusion in rats. Spine 17:701-707, 1992. Bibliographic Links [Context Link]

31. Jaroma HJ, Ritsila VA: Effect of diffusion chamber pore size on differentiation and proliferation of periosteal cells. Clin Orthop 236:258-264, 1988. Bibliographic Links [Context Link]

32. Jingushi S, Joyce ME, Bolander ME: Genetic expression of extracellular matrix proteins correlates with histologic changes during fracture repair. J Bone Miner Res 7:1045-1055, 1992. Bibliographic Links [Context Link]

33. Johnson KA, Howlett CR, Bellenger CR, Armati-Gulson P: Osteogenesis by canine and rabbit bone marrow in diffusion chambers. Calcif Tissue Int 42:113-118, 1988. Bibliographic Links [Context Link]

34. Johnstone B, Yoo JU, Barry F: In vitro chondrogenesis of bone marrow derived mesenchymal cells. Trans Orthop Res Soc 21:65, 1996. [Context Link]

35. Kasugai S, Todescan Jr R, Negata T, et al: Expression of bone matrix proteins associated with mineralized tissue formation by adult rat bone marrow cells in vitro: Inductive effects of dexamethasone on the osteoblastic phenotype. J Cell Physiol 147:111-120, 1991. Bibliographic Links [Context Link]

36. Katagiri T, Yamaguchi A, Ikeda T, et al: The nonosterogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate into osteoblastic cells by recombinant human bone morphogenetic protein-2. Biochem Biophys Res Commun 172:295-299, 1990. Bibliographic Links [Context Link]

37. Klagsbrun M: The fibroblast growth factor family: Structural and biological properties. Prog Growth Factor Res 1:207-235, 1989. Bibliographic Links [Context Link]

38. Leboy PS, Beresford JN, Devlin C, Owen ME: Dexamethasone induction of osteoblastic mRNAs in rat marrow stromal cell cultures. J Cell Physiol 146:370-378, 1991. [Context Link]

39. Lian JB, Stein GS: The developmental stages of osteoblast growth and differentiation exhibit selective responses of genes to growth factors (TGF beta 1) and hormones (vitamin D and glucocorticoids). J Oral Implantol 19:95-105, 1993. Bibliographic Links [Context Link]

40. Lindholm TS, Nilsson OS, Lindholm TC: Extraskeletal and intraskeletal new bone formation induced by demineralized bone matrix combined with bone marrow cells. Clin Orthop 171:251-255, 1982. Bibliographic Links [Context Link]

41. Lindholm TS, Ragni P, Lindholm TC: Response of bone marrow stroma cells to demineralized cortical bone matrix in experimental spinal fusion in rabbits. Clin Orthop 230:296-302, 1988. Bibliographic Links [Context Link]

42. Lovell TP, Dawson EG, Nilsson OS, Urist MR: Augmentation of spinal fusion with bone morphogenetic protein in dogs. Clin Orthop 243:266-274, 1989. Bibliographic Links [Context Link]

43. Maniatopoulos C, Sodek J, Melcher AH: Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats. Cell Tissue Res 254:317-330, 1988. Bibliographic Links [Context Link]

44. Marden LJ, Hollinger JO, Chaudhari A, et al: Recombinant human bone morphogenetic protein-2 is superior to demineralized bone matrix in repairing craniotomy defects in rats. J Biomed Mater Res 28:1127-1138, 1994. Bibliographic Links [Context Link]

45. McKibbin B: The biology of fracture healing in long bone. J Bone Joint Surg 60B:150-162, 1978. [Context Link]

46. Nakahara H, Bruder SP, Haynesworth SE, et al: Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. Bone 11:181-188, 1990. Bibliographic Links [Context Link]

47. Nakahara H, Goldberg VM, Caplan AI: Culture-expanded periosteal-derived cells exhibit osteochondrogenic potential in porous calcium phosphate ceramics in vivo. Clin Orthop 276:291-298, 1992. [Context Link]

48. Noble BS, Dean V, Loveridge N, Thomson BM: Dextran sulfate promotes the rapid aggregation of porcine bone marrow stromal cells. Bone 17:375-382, 1995. Full Text Bibliographic Links [Context Link]

49. Noff D, Pitaru S, Savion N: Basic fibroblast growth factor enhances the capacity of bone marrow cells to form bone-like nodules in vitro. FEBS Lett 250:619-621, 1989. Full Text Bibliographic Links [Context Link]

50. Oikarinen J: Experimental spinal fusion with decalcified bone matrix and deep-frozen allogeneic bone in rabbits. Clin Orthop 162:210-218, 1982. Bibliographic Links [Context Link]

51. Owen ME, Friedenstein AJ: Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Found Symp 136:42-60, 1988. Bibliographic Links [Context Link]

52. Paley D, Young MC, Wiley AM, et al: Percutaneous bone marrow grafting of fractures and bony defects: An experimental study in rabbits. Clin Orthop 208:300-312, 1986. Bibliographic Links [Context Link]

53. Pitaru S, Kotev-Emeth S, Noff D, et al: Effect of basic fibroblast growth factor on the growth and differentiation of adult stromal bone marrow cells: Enhanced development of mineralized bone-like tissue in culture. J Bone Miner Res 8:919-929, 1993. Bibliographic Links [Context Link]

54. Quarto R, Thomas D, Liang CT: Bone progenitor cell deficits and age-associated decline in bone repair capacity. Calcif Tissue Int 56:123-129, 1995. Bibliographic Links [Context Link]

55. Reddi AH, Cunningham NS: Initiation and promotion of bone differentiation by bone morphogenetic proteins. J Bone Miner Res 8(Suppl):S499-502, 1993. Bibliographic Links [Context Link]

56. Rickard DJ, Sullivan TA, Shenker BJ, et al: Induction of rapid osteoblast differentiation in rat bone marrow stromal cell cultures by dexamethasone and BMP-2. Dev Biol 161:218-228, 1994. Full Text Bibliographic Links

57. Sandhu HS, Kanim LEA, Kabo JM, et al: Evaluation of rhBMP-2 with an OPLA carrier in a canine posterolateral (transverse process) spinal fusion model. Spine 20:2669-2682, 1995. Bibliographic Links [Context Link]

58. Sandhu HS, Kanim LEA, Kabo JM, et al: Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 21:2115-2122, 1996. Ovid Full Text Bibliographic Links [Context Link]

59. Schimandle JH, Boden SD, Hutton WC: Experimental spinal fusion with recombinant human bone morphogenetic protein-2. Spine 20:1326-1337, 1995. Bibliographic Links [Context Link]

60. Stevenson S, Cunningham N, Toth J, et al: The effect of osteogenin (a bone morphogenetic protein) on the formation of bone in orthotopic segmental defects in rats. J Bone Joint Surg 76A:1676-1687, 1994. Ovid Full Text Bibliographic Links [Context Link]

61. Takagi K, Urist MR: The role of bone marrow in bone morphogenetic protein-induced repair of femoral massive diaphyseal defects. Clin Orthop 171:224-231, 1982. Bibliographic Links [Context Link]

62. Topping RE, Bolander ME, Galian G: Type X collagen in fracture callus and the effects of experimental diabetes. Clin Orthop 308:220-228, 1994. Bibliographic Links [Context Link]

63. Urist MR, Dawson E: Intertransverse process fusion with the aid of chemosterilized autolyzed antigen-extracted allogenic (AAA) bone. Clin Orthop 154:97-113, 1981. Bibliographic Links [Context Link]

64. Vacanti CA, Kim W, Upton J, et al: The efficacy of periosteal cells compared to chondrocytes in the tissue engineered repair of bone defects. Tissue Eng 1:301-308, 1995. [Context Link]

65. Wang EA, Rosen V, D'Alessandro JS, et al: Recombinant human bone morphogenetic protein induced bone formation. Proc Natl Acad Sci USA 87:2220-2224, 1990. Bibliographic Links

66. Werntz JR, Lane JM, Burstein AH, et al: Qualitative and quantitative analysis of orthotopic bone regeneration by marrow. J Orthop Res 14:85-93 1996. Bibliographic Links [Context Link]

67. Yao KL, Todescan Jr R, Sodek J: Temporal changes in matrix protein synthesis and mRNA expression during mineralized tissue formation by adult rat bone marrow cells in culture. J Bone Miner Res 9:231-240, 1994. Bibliographic Links [Context Link]

68. Yasko AW, Lane JM, Fellinger U, et al: The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). J Bone Joint Surg 74A:659-671, 1992. [Context Link]

69. Young RW: Cell proliferation and specialization during endochondral osteogenesis in young rats. J Cell Biol 14:357-370, 1962. [Context Link]

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