Chapter 1
Clinical Applications of Stem Cells for Bone Repair

Wasim S. Khan, Philip Pastides, and David Marsh

1.1 Introduction

Regeneration involves replacement of old tissue with new tissue. It occurs readily in the embryo but is slow in most adult tissue. This may be because of the relatively large number of undifferentiated progenitor cells in the embryo compared with adults. Repair mechanisms in post-embryonic tissue, other than bone, result in scar formation instead of tissue regeneration. Repair is more rapid and designed for survival. It involves the inflammatory cell cascade followed by matrix deposition and the remodelling process which attempts to regenerate damaged tissue.

Bone is continually remodelled as a result of the balance between the activities of the osteoclasts and the osteoblasts. Because of the potential of bone to spontaneously regenerate, most bone lesions, such as fractures, heal well with conventional therapy or surgery. The osteogenic process that commences after the inflammatory phase, under the influence of bone-derived bioactive factors, is initiated by precursor cells from the periosteum adjacent to the fracture site. This generates hard callus by intramembranous bone formation. A bone graft or substitute is often required to assist in orthopaedic surgery healing of a large traumatic or post-surgical defect and of osseous congenital deformities. The majority of bone formation however is by enchondral ossification of the soft callus that appears after infiltrated mesenchymal cells are induced to chondrogenesis. This improved understanding of repair and regeneration has helped with the development of orthopaedic tissue engineering (Kruyt et al. 2004).

W.S. Khan (✉) • P. Pastides • D. Marsh
Royal National Orthopaedic Hospital, University College London Institute for Orthopaedics and Musculoskeletal Sciences, Stanmore HA7 4LP, UK
e-mail: wasimkhan@doctors.org.uk

P. Van Pham (ed.), *Bone and Cartilage Regeneration*, Stem Cells in Clinical Applications, DOI 10.1007/978-3-319-40144-7_1

Current surgical treatment of large bone defects falls into two groups: Illizarov method or bone transport and bone graft transplant (auto-, allo-, xeno-grafts, different biomaterial implants). The Illizarov technique entails an osteotomy followed by bone distraction allowing regeneration of bone. The disadvantages include long recovery periods and a high complication rate. The clinical gold standard for bone repair is an autologous graft that is effective, but this is limited by the availability of sufficient donor tissue and donor site morbidity. As for graft transplants, vascularised autografts are presently mostly used, e.g. autografting cancellous bone applying vascularised grafts of the fibula and iliac crest (Perry 1999). The disadvantages include problems related to anatomical limitations, graft integration and donor site morbidity, including infection, haematoma and limitation of size of defect reconstruction.

Tissue engineering has been defined as the application of scientific principles to the design, construction, modification and growth of living tissue using biomaterials, cells and growth factors, either alone or in combination (Langer and Vacanti 1993; Khan et al. 2009, 2012a). In essence, three elements are central in tissue engineering; stem or precursor cells; an appropriate biological scaffold and growth factors. All three are discussed in detail below. It generally involves the use of cells with a matrix or scaffold that guides the cells during tissue repair or regeneration. The use of more undifferentiated cell types such as stem cells or early mesenchymal progenitors that retain multi-lineage and self-renewal potential is preferable to the use of terminally differentiated cells. The scaffold could be natural or biosynthetic. Cells harvested from donor tissues, including adult stem cells, can be expanded in culture and associated with resorbable biomaterials to form a scaffold. The cells can be stimulated by specific bioactive molecules called growth factors (Kanitkar et al. 2011). The cells can also be genetically modified via genomic insertion of a new healthy copy of a gene before expansion and subsequent tissue reconstitution. To date the use of gene technology has not been applied to humans; the cells could be stimulated in vitro to form tissue for future re-implantation in vivo. In vitro, this process may be facilitated by the use of a bioreactor that provides the appropriate environment to allow the process (Oragui et al. 2011; Mabvuure et al. 2012).

A tissue engineering approach to treat skeletal defects involves the use of osteoconductive biomaterial scaffolds with osteogenic cell populations and osteoinductive bioactive factors. A possible tissue engineering approach for bone repair is to use autologous bone marrow stem cells (BMSC) loaded onto a scaffold (Cancedda et al. 2003). The three constituents are discussed below.

1.2 Mesenchymal Stem Cells

Differentiated cells released from adult tissue exhibit a limited proliferation capacity. This has limitations for their expansion in culture and in vitro reconstruction of tissue. Culturing undifferentiated cells (stem cells or progenitor cells) that have a

higher proliferative capacity is more promising (Khan et al. 2012b). Differentiation of these cells can be obtained in vitro by changing the culture conditions after their expansion or by providing a new physiological micro-environment in the transplant area in vivo (Thanabalasundaram et al. 2012).

A stem cell is a cell from the embryo, foetus or adult that, under certain conditions, can reproduce for long periods. It can also give rise to specialized cells of body tissues and organs. The use of stem cells from the embryo or foetus has many ethical considerations whereas the use of adult stem cells is generally well accepted by society. An adult stem cell is an undifferentiated or unspecialized cell present in differentiated tissue, which renews itself; and becomes specialized to yield all of the cell types of the tissue from which it was originated. Their progeny includes both new stem cells and committed progenitors with a more restricted differentiation potential. These progenitor cells in turn give rise to more differentiated cell types. The advantages of using stem cells rather than differentiated cells are a higher proliferative capacity, a higher regenerative potential over time and the ability to allow revascularization of the avascular scaffold (Shekkeris et al. 2012). Cells with osteoprogenitor features have been isolated from several tissues including periosteum, bone marrow, adipose tissue, and even the umbilical cord and placenta (Longo et al. 2012; Mohal et al. 2012). The choice of source depends on accessibility, frequency of cells and information of a particular cell system (Fossett et al. 2012).

Research suggests that stem cells derived from bone marrow (BMSC) can be expanded for a significant number of cell doublings without cell senescence. In vitro multidifferentiation potentials are gradually lost on expansion (Cancedda et al. 2003). The harvest of bone marrow samples is an easy and relatively safe procedure. The bone marrow is a reservoir of multipotent stem cells for mesenchymal tissues. These multipotential stromal stem cells can differentiate into fibroblastic, osteogenic, adipogenic and reticular cells (Bianco et al. 2001). A large number of BMSCs can be obtained in culture. In addition, human bone marrow osteoprogenitor cells can be isolated and enriched using monoclonal antibodies as selective markers, such as STRO-1 from a CD34+ fraction, SB-10 (reacting with ALCAM), SH-2 (reacting with CD105) and HOP-26 (reacting with CD63) (Oreffo and Triffitt 1999; Stewart et al. 1999; Partridge and Oreffo 2004). FGF-2 supplementation to the culture medium promotes cell proliferation and maintains their multi-lineage potential during expansion (Cancedda et al. 2003).

Intraoperative adult stem cells technologies are being developed to enhance bone repair in delayed or non-union fractures as shown by Muschler et al. in 2003 Muschler et al. (2003). One in 23,000 adult bone marrow cells is an osteogenic precursor cell. These cells can potentially be separated by selective cell absorption in the operating theatre making viable implants for immediate surgical use. These cells can be combined with a suitable scaffold and used as an alternative to conventional bone autograft. The transplanted osteogenic stem cells can immediately begin to proliferate and lay down new bone matrix without removing the old matrix present in the autograft. The development of these cell based technologies may result in decreased use of conventional bone banks that use dead bone to induce new bone formation.

1.3 Scaffolds

Mesenchymal stem cells alone are unlikely to be sufficient for bone regeneration. Although marrow injections are simple and provide a reduced risk of morbidity, for large skeletal defects, a scaffold of appropriate shape, size and mechanical competence is required for fracture repair. The use of the scaffold or matrix is not only in controlling growth factor and cell delivery but also to provide a structural template to fill the tissue lesion. These could be naturally occurring or synthetic polymers or bioceramics. Biodegradable scaffolds provide the initial structure and stability for tissue formation but degrade as tissue forms, providing room for matrix deposition and tissue growth. They can be used alone or in combination with growth factors or osteoconductive materials.

The scaffold aims to mimic the extracellular matrix in a regenerating bone environment. It has to be informative to the cells as well as provide mechanical support. A biomaterial should easily integrate with the adjacent bone and favour new tissue ingrowth (osteoconduction). It should allow colonization by the host blood vessels, be biocompatible and resorbable.

Polymers include collagen that can be prepared in solution or shaped into membrane films, threads, sponges and acidic hydrogels (Mafi et al. 2012). It is derived from xenogenic sources and purification techniques are used to eliminate the immunogenic telopeptides. The primary obstacle to their use is the possibility of xenozoonoses (Lee et al. 2001). Heparin-coating fibrin hydrogels can be used to slowly and regularly deliver growth factors with heparin binding affinity such as FGF-2. Alginates extracted from brown algae form a brown lattice hydrogel. It has large average mesh size allowing easy diffusion of macromolecules. Hyaluronic acid binds specifically to proteins. Its stability is increased by partial esterification making it particularly suitable for peptide release or protein delivery (Grigolo et al. 2001).

Synthetic polymers include poly lactic acid (PLA), polyglycolic acid (PGA) and their copolymer polylactic-co-glycolic acid (PLGA). They allow a better control of physicochemical properties and delivery kinetics. They also reduce the risk of potential biohazardous complications. The disadvantages are the induction of some immune or inflammatory response after implantation. These polymers are currently used for a number of orthopaedic devices including suture anchors and interference screws. Other biodegradable materials for bone tissue engineering include Degrapolfoam and Polyactive that support bone cell adhesion and proliferation (Sakkers et al. 2000). Surface eroding polymers such as polyortho-esters may have advantages in load bearing bone applications as only the surface of these materials degrades leaving the bulk the mechanical strength (Andriano et al. 1999).

Bioceramics act as a pre-existing bone surface on which bone cells deposit new bone matrix. The best results to date have been with porous bioceramics and BMSC. Bioceramics made from hydroxyapatite and tricalcium phosphate are used for bone repair. They have osteoconductive properties and the ability to integrate with bone tissue. They are not themselves osteoinductive and are resorbed relatively slow. Their resorbability can be increased by increasing the concentration of tricalcium phosphate. The production of porous scaffolds makes the internal architecture similar

to that of cancellous bone. The advantage of such materials include a large surface available for tissue regeneration and cell delivery and a favourable micro-environmental effect due to the presence of a mineralized matrix (LeGeros 2002). Problems include biodegradability and inflammatory and immunological reactions (Oreffo and Triffitt 1999).

Alternative scaffolds can be derived from cadavers or animal skeletons; natural scaffolds can be derived from coral cytoskeleton. Mesenchymal stem cells mixed with coral implants have been shown to stimulate bone regeneration, achieve bone regeneration and clinical union in an animal model (Petite et al. 2000). Biomimetic material chemistry attempts to reproduce the complex structures that occur in nature (such as coral, nacre, calcite shells and sea urchin spines) in synthetic systems and generate accurate and specific biomaterials. They potentially mimic many roles of the extracellular matrix by providing biological cues for cell–matrix interactions promoting tissue growth. They are modified with bioactive molecules and can be used as tissue engineering scaffolds. More information on immunoreactivity and biocompatibility will be needed before clinical evaluation (Shin et al. 2003).

Smart materials, for example Arg-Gly-Asp (RGD), sequence peptides involved in integrin mediated cell adhesion and can be incorporated onto the scaffold surface to enhance cell adhesion and spreading (Quirk et al. 2001). Drug delivery techniques such as entrapment within a hydrogel matrix allow growth factor to be released in a controlled fashion from the scaffold and aid in tissue regeneration (Whitaker et al. 2001). This strategy has been applied in bone tissue engineering. Growth factors such as recombinant human bone morphogenetic protein-2, basic fibroblast growth factor and vascular endothelial growth factor have all been successfully incorporated into a hydrogel prior to in vivo implantation (Lee et al. 2000). The ultimate goal may be the introduction of calcium based scaffolds that can gradually degrade at the same rate of new bone formation.

1.4 Growth Factors

Growth factors are cytokines that are secreted by many cell types and function as signalling molecules. Members of the TGF beta family, notably bone morphogenetic proteins (BMPs), are particularly relevant to skeletal tissue engineering. Other agents known to induce bone formation include FGF, PDGF and IGF-1, Indian sonic hedgehog and parathyroid hormone. One function of BMP is to induce the differentiation of undifferentiated mesenchymal cells into chondrogenic and osteogenic cells and to promote their proliferation. BMPs have a role in bone development and are thus heavily incorporated into tissue engineered scaffolds and delivery systems (Boden 1999).

The isolation of growth factors such as TGF beta 3 and its analogues such as BMP 2 and 7 has led to an enhanced and accelerated repair and replacement of bony lesions. Bone induction to assist and enhance bone deposition and repair was first introduced by Marshall Urist in 1965 Urist (1965), and led to the isolation of BMP. Human cDNA BMP 7 was cloned in 1990 and a recombinant human form followed. It was shown to

induce bone formation in animals by stimulating precursor MSCs. Recombinant human BMPs (rhBMPs) have been commercially available for over a decade but their bioavailability, bioactivity and costs have limited their clinical uptake. There are three methods by which BMPs can be used in bone tissue engineering: (a) cell therapy (b) gene therapy (c) cytokine therapy.

Earlier examples include the use of porous PLGA scaffolds with high molecular weight hyaluronic acid for rhBMP-2 delivery (Brekke and Toth 1998). TGF beta 1 has been shown to stimulate the three-dimensional cellular development of human bone ex vivo (Kale et al. 2000).

A prospective randomized controlled trial of 450 patients concluded that the clinical use of rhBMP-2 as an adjunct to standard management of long bone fractures was safe, well tolerated and resulted in an earlier functional recovery (Govender et al. 2002).

1.5 Gene Therapy

Gene therapy is the science of transfer of genetic material into individuals for therapeutic purposes by altering cellular function or structure at the molecular level. The ability to transfer genes into multipotent mesenchymal stem cells has many applications. Developments in gene technology offer the possibility of genetic modification of isolated and expanded cells to produce populations of progenitor cells overexpressing selected signalling molecules. The various techniques and methods currently available to enable gene transfer into a target population include viral methods (transduction) and nonviral methods (transfection). Viral delivery systems used for bone engineering include retroviral and adenoviral systems. The advantages of viral expression of genes are a high efficiency of transduction (50–100 %). The disadvantages include the potential for mutagenesis, carcinogenesis and an evoked immune response. Nonviral gene delivery makes use of lipopolyfection reagents such as liposomes, cationic lipids or cationic polymers complexed with a foreign DNA for transfection. Alternatively they can use physical methods such as microinjection, gene gun delivery or the use of uncomplexed plasmid DNA. The nonviral methods are safer but less efficient and some can cause immunological reactions (Partridge and Oreffo 2004). Another approach is to use matrices for gene or protein delivery. These provide a stable and sustained release of allogenic cortical bone and synthetic substances (Braddock et al. 2001).

Gene delivery can be direct in vivo or indirect ex vivo. The direct method involves transferring the genetic material into the target somatic cell in vivo. This is technically simpler to perform in a clinical setting. The indirect technique involves removal of cells from the patient, genetic modifications of the cells ex vivo and return of the cells to the patient. This is technically more complex but is relatively safer and allows for selection of cells for gene expression (Wu et al. 2003). Lieberman et al. have shown that regional cell and gene therapy using BMP-2 with bone marrow derived cells resulted in the repair of segmental bone defects in rats. Brietbant et al. have cultured periosteal cells retrovirally transduced with BMP-7 in

a polyglycolic acid (PGA) scaffold to treat critical sized calvarial defects in rabbits. Furthermore, Olmsted et al. (2001) have indicated the potential to generate human bone marrow stromal cells expressing BMP-2 by adenoviral infection.

1.6 Conclusion

The aim of all these techniques is to provide the reconstructed segment with appropriate initial mechanical properties while encouraging new bone formation (Chimutengwende-Gordon and Khan 2012). Bone formation by BMSC transplanted into small animals was first demonstrated by Friedenstein in 1966. Implanting BMSC combined with 3D mineralized bioceramic scaffolds subcutaneously into immunodeficient mice can be used to assess bone formation. Autologous BMSC and bioceramic composites have been used to repair full thickness defects within the tibial diaphysis of sheep. Gross morphology, radiographs and histology show complete integration of ceramic with bone and good functional recovery. Culture expanded bone marrow cells can heal a segmental bone defect following re-implantation (Kadiyala et al. 1997) and can give rise to osteogenic tissue within diffusion chambers in a variety of animal species (Gundle et al. 1995). Similar results with carol scaffold and hydroxyapatite and beta tricalcium phosphate scaffold have also been described.

Autologous osteoprogenitor cells were isolated from the bone marrow of patients with lower limb bone detects of between 4 and 7 cm for whom a traditional therapeutic alternative was difficult or had previously failed and expanded in vitro were delivered in vivo via a microporous hydroxyapatite scaffold. Stability was provided via external fixation methods. By the second month, abundant callus formation along the implant region and good integration at the bone interface were observed. No major complication was observed. All patients recovered limb function in 6–12 months. In cases where bone defects occur in positions requiring dynamic strength, such as long bones of the legs, an alternative to using an external fixator is to use solid implant with good affinity to bone. For instance a titanium implant with a porous surface on which BMP and polymer composites are placed has been shown to allow bone formation to occur on the composite material used.

References

Andriano KP, Tabata Y, Ikada Y, Heller J. In vitro and in vivo comparison of bulk and surface hydrolysis in absorbable polymer scaffolds for tissue engineering. J Biomed Mater Res. 1999;48(5):602–12.

Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells. 2001;19(3):180–92.

Boden SD. Bioactive factors for bone tissue engineering. Clin Orthop Relat Res. 1999(367 Suppl):S84–94.

Braddock M, Houston P, Campbell C, Ashcroft P. Born again bone: tissue engineering for bone repair. News Physiol Sci. 2001;16:208–13.
Brekke JH, Toth JM. Principles of tissue engineering applied to programmable osteogenesis. J Biomed Mater Res. 1998;43(4):380–98.
Cancedda R, Bianchi G, Derubeis A, Quarto R. Cell therapy for bone disease: a review of current status. Stem Cells. 2003;21(5):610–9.
Chimutengwende-Gordon M, Khan WS. Advances in the use of stem cells and tissue engineering applications in bone repair. Curr Stem Cell Res Ther. 2012;7(2):122–6.
Fossett E, Khan WS, Longo UG, Smitham PJ. Effect of age and gender on cell proliferation and cell surface characterization of synovial fat pad derived mesenchymal stem cells. J Orthop Res. 2012;30(7):1013–8.
Govender S, et al. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am. 2002;84-A(12):2123–34.
Grigolo B, Roseti L, Fiorini M, Fini M, Giavaresi G, Aldini NN, Giardino R, Facchini A. Transplantation of chondrocytes seeded on a hyaluronan derivative (hyaff-11) into cartilage defects in rabbits. Biomaterials. 2001;22(17):2417–24.
Gundle R, Joyner CJ, Triffitt JT. Human bone tissue formation in diffusion chamber culture in vivo by bone-derived cells and marrow stromal fibroblastic cells. Bone. 1995;16(6):597–601.
Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant. 1997;6(2):125–34.
Kale S, Biermann S, Edwards C, Tarnowski C, Morris M, Long MW. Three-dimensional cellular development is essential for ex vivo formation of human bone. Nat Biotechnol. 2000;18(9):954–8.
Kanitkar M, Tailor HD, Khan WS. The use of growth factors and mesenchymal stem cells in orthopaedics. Open Orthop J. 2011;5:271.
Khan WS, Malik A, Hardingham T. Stem cell applications and tissue engineering approaches in surgical practice. J Perioper Pract. 2009;19(4):130–5.
Khan WS, Pastides P, Marsh DR. Tissue engineering approaches for bone repair. In: Khan WS, Pastides P, editors. Tissue engineering and the musculoskeletal system: a limitless cure? New York: Nova Science Publishers; 2012a. p. 31–46.
Khan WS, Adesida AB, Tew SR, Longo UG, Hardingham TE. Fat pad-derived mesenchymal stem cells as a potential source for cell-based adipose tissue repair strategies. Cell Prolif. 2012b;45(2):111–20.
Kruyt MC, van Gaalen SM, Oner FC, Verbout AJ, de Bruijn JD, Dhert WJ. Bone tissue engineering and spinal fusion: the potential of hybrid constructs by combining osteoprogenitor cells and scaffolds. Biomaterials. 2004;25(9):1463–73.
Langer R, Vacanti JP. Tissue engineering. Science. 1993;260(5110):920–6.
Lee KY, Peters MC, Anderson KW, Mooney DJ. Controlled growth factor release from synthetic extracellular matrices. Nature. 2000;408(6815):998–1000.
Lee CH, Singla A, Lee Y. Biomedical applications of collagen. Int J Pharm. 2001;221(1–2):1–22.
LeGeros RZ. Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthop Relat Res. 2002;395:81–98.
Longo UG, Loppini M, Berton A, La Verde L, Khan WS, Denaro V. Stem cells from umbilical cord and placenta for musculoskeletal tissue engineering. Curr Stem Cell Res Ther. 2012;7(4):272–81.
Mabvuure N, Hindocha S, Khan WS. The role of bioreactors in cartilage tissue engineering. Curr Stem Cell Res Ther. 2012;7(4):287–92.
Mafi P, Hindocha S, Mafi R, Khan WS. Evaluation of biological protein-based collagen scaffolds in cartilage and musculoskeletal tissue engineering-A systematic review of the literature. Curr Stem Cell Res Ther. 2012;7(4):302–9.
Mohal M, Tailor HD, Khan WS. Sources of adult mesenchymal stem cells and their applicability for musculoskeletal applications. Curr Stem Cell Res Ther. 2012;7(2):103–9.

Muschler GF, Nitto H, Matsukura Y, Boehm C, Valdevit A, Kambic H, Davros W, Powell K, Easley K. Spine fusion using cell matrix composites enriched in bone marrow-derived cells. Clin Orthop Relat Res. 2003;407:102–18.

Olmsted EA, Blum JS, Rill D, Yotnda P, Gugala Z, Lindsey RW, Davis AR. Adenovirus-mediated BMP2 expression in human bone marrow stromal cells. J Cell Biochem. 2001;82(1):11–21.

Oragui E, Nannaparaju M, Khan WS. The role of bioreactors in tissue engineering for musculoskeletal applications. Open Orthop J. 2011;5:267.

Oreffo RO, Triffitt JT. Future potentials for using osteogenic stem cells and biomaterials in orthopedics. Bone. 1999;25(2 Suppl):5S–9.

Partridge KA, Oreffo RO. Gene delivery in bone tissue engineering: progress and prospects using viral and nonviral strategies. Tissue Eng. 2004;10(1–2):295–307.

Perry CR. Bone repair techniques, bone graft, and bone graft substitutes. Clin Orthop Relat Res. 1999;360:71–86.

Petite H, Viateau V, Bensaid W, Meunier A, de Pollak C, Bourguignon M, Oudina K, Sedel L, Guillemin G. Tissue-engineered bone regeneration. Nat Biotechnol. 2000;18(9):959–63.

Quirk RA, Chan WC, Davies MC, Tendler SJ, Shakesheff KM. Poly(L-lysine)-GRGDS as a biomimetic surface modifier for poly(lactic acid). Biomaterials. 2001;22(8):865–72.

Sakkers RJ, Dalmeyer RA, de Wijn JR, van Blitterswijk CA. Use of bone-bonding hydrogel copolymers in bone: an in vitro and in vivo study of expanding PEO-PBT copolymers in goat femora. J Biomed Mater Res. 2000;49(3):312–8.

Shekkeris AS, Jaiswal PK, Khan WS. Clinical applications of mesenchymal stem cells in the treatment of fracture non-union and bone defects. Curr Stem Cell Res Ther. 2012;7(2):127–33.

Shin H, Jo S, Mikos AG. Biomimetic materials for tissue engineering. Biomaterials. 2003;24(24):4353–64.

Stewart K, Walsh S, Screen J, Jefferiss CM, Chainey J, Jordan GR, Beresford JN. Further characterization of cells expressing STRO-1 in cultures of adult human bone marrow stromal cells. J Bone Miner Res. 1999;14(8):1345–56.

Thanabalasundaram G, Arumalla N, Tailor HD, Khan WS. Regulation of differentiation of mesenchymal stem cells into musculoskeletal cells. Curr Stem Cell Res Ther. 2012;7(2):95–102.

Urist MR. Bone: formation by autoinduction. Science. 1965;150(698):893–9.

Whitaker MJ, Quirk RA, Howdle SM, Shakesheff KM. Growth factor release from tissue engineering scaffolds. J Pharm Pharmacol. 2001;53(11):1427–37.

Wu D, Razzano P, Grande DA. Gene therapy and tissue engineering in repair of the musculoskeletal system. J Cell Biochem. 2003;88(3):467–81.