Published in final edited form as:

BioDrugs. 2012 August 1; 26(4): 245–256. doi:10.2165/11631680-000000000-00000.

Recommendations and Considerations for the Use of Biologics in Orthopedic Surgery

Stefan Zwingerberger^{1,2}, Christophe Nich^{1,3,4}, Roberto D. Valladares¹, Zhenyu Yao¹, Maik Stiehler², and Stuart B. Goodman¹

¹Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, CA, USA

²Department of Orthopaedics, University Hospital Carl Gustav Carus at Technical University Dresden, Dresden, Germany

³Laboratoire de Biomécanique et Biomatériaux Ostéo-Articulaires, UMR CNRS 7052, Faculté de Médecine, Université Paris 7, Paris, France

⁴Department of Orthopaedic Surgery, European Teaching Hospital, Assistance Publique, Hôpitaux de Paris, Université Paris 5, Paris, France

Abstract

Reconstruction of extensive bone defects remains technically challenging and has considerable medical and financial impact on our society. Surgical procedures often require a bone/substitute graft to enhance and accelerate bone repair. Bone autografts are associated with morbidity related to bone harvesting and are limited in quantity. Alternatively, bone allografts expose the patient to the risk of transmission of infectious disease. Synthetic bone graft substitutes, such as calcium sulfates, hydroxyapatite, tricalcium phosphate, and combinations, circumvent some of the disadvantages of auto- and allografts, but have limited indications. Biomedical research has made possible the stimulation of the body's own healing mechanisms, either by delivering exogenous growth factors locally, or by stimulating their local production by gene transfer. Among all known factors having osteoinductive properties, only two bone morphogenetic proteins (for specific indications) and demineralized bone matrix have been approved for clinical use. In addition, ongoing research is exploring the efficacy of cell therapy and tissue engineering. The present report examines the composition, biological properties, indications, clinical experience and regulations of several of the biotherapeutics employed for bone reconstruction.

1. Introduction

Bone tissue has a remarkable ability for regeneration and repair as part of physiological remodeling, or in response to injury. In some situations, bone repair cannot occur spontaneously because of adverse local conditions (vascular injury, infection, etc.), a bone defect has reached a critical size, systemic causes, or combinations. Numerous procedures

have been described to treat these complex issues. Distraction osteogenesis and bone transport are technically demanding and have high complication rates. A number of surgical procedures to augment bone regeneration imply the use of 'biologic support' in the form of a bone graft or substitute, either natural or synthetic. Today, more than 500 000 bone-grafting procedures are performed in the US each year, most of them being related to spine fusion. The 'gold standard' of bone graft remains autogenous bone graft (autograft). Only autograft achieves the most desirable properties of a bone graft material, including osteoconduction (the matrix), osteoinduction (growth factors), and osteogenesis (osteoprogenitor or osteogenic cells).^[1] However, bone autografts need an additional surgical site, with potential associated morbidity, and are limited in quantity. Allografts circumvent some of the issues relative to autograft, but they present concerns as well, such as the risk of transmission of infectious disease,^[2] immunological reactions by the recipient, loss of biologic and mechanical properties due to their processing, increased costs, and availability. Improved biological safety is a desirable characteristic of synthetic bone grafts. Approximately 60% of the synthetic bone graft substitutes currently available involve ceramics. These include calcium sulfates, hydroxyapatite, tricalcium phosphate, or combinations thereof. Recent generations of bone substitutes have introduced the potential for synthetic bone grafts to promote biologic repair, and to provide support for treatment, such as antibacterials or bone morphogenetic proteins. The drawbacks and potential complications related to the use of allo- and autograft, and the limited indications for synthetic bone grafts, have facilitated the progress toward a biologic alternative. Exogenous treatments enable the enhancement and acceleration of bone healing, and include bone growth factors and demineralized bone matrix (DBM). This paper reviews the current knowledge and field of application of biological options available for promoting bone repair, including natural and synthetic bone grafts, growth factors, and gene- and cell-based strategies.

See figure 1 for an overview of biologics in orthopedic surgery.

2. Osteoconductive Bone Substitutes: Biologics as Scaffolds

2.1 Bone Allograft

Bone allografts are obtained from human cadavers or from living donors (e.g. discarded femoral heads). They act as tridimensional biological scaffolds, which support the direct growth of bone over their surface (osteoconduction), and can revascularize and incorporate into the host bed. Allografts have the optimal porosity and microstructure with reference to human bone. Their incorporation is driven by the process of 'creeping substitution'. Both intramembranous and endochondral bone formation occurs on graft surfaces.^[3] Mostly, persistent dead trabecula will remain on the innermost layer of the graft bed for many years.^[4–8] Bone allografts should be employed mainly in mechanically protected environments.

The expanding demand for bone allograft is mainly driven by the growing number of revision arthroplasties. Impaction grafting has been shown to restore satisfactory socket stability, with implant survival rate ranging from 85% to 95% at 10–12 years.^[9] When combined defects are encountered, allograft is usually used in conjunction with an anti-protrusion cage construct, which protects the grafted material.^[10–12] On the femoral side,

large cavitory defects can be treated with packed particulate bone graft in association with cemented implants.^[13] Massive proximal femoral defects require both restoration of bone stock and mechanical stability. In these cases, a long-stemmed metallic femoral component cemented into a proximal femoral allograft may be indicated. Technically, the protruding distal stem of this so-called allograft prosthesis composite (APC) is inserted into the host's remaining distal femur. The APC restores femoral bone stock, offers optimal biomechanical properties, and allows reattachment of the hip abductor muscles. Globally, these techniques lead to a significant improvement in function and satisfactory survival rates, estimated to be 81% at a mean of 8.1 years.^[14] However, complication rates are reportedly high, notably infection, instability, failure of the APC, and nonunion.^[15] In revision total knee arthroplasty, large defects can be addressed with bulk grafts, associated with metallic augments. Bulk or massive allografts are recommended in conjunction with long-stemmed components, to offload mechanical stress from the graft. These techniques provided 80–93% survivorship rates at intermediate terms,^[16–18] but some concerns have arisen after a 5-year implantation period with a consistent drop in survival.^[16] In addition, rates of complications are reportedly high, despite consistent improvement in function.^[16,18,19] In posterior spinal arthrodesis, allografts are associated with lower fusion rates than autograft,^[20] although clinical results appear to be comparable.^[21]

2.2 Synthetic Bone Grafts

2.2.1 Calcium Sulfate (Plaster of Paris)—Plaster of Paris is a bioabsorbable ceramic, composed of dihydrated calcium sulfate (CaSO_4). Calcium sulfate is biocompatible, bioactive, and biodegrades after 4–8 weeks. It is characterized by a lack of macroporosity, which implies that no osteoconduction can occur within it. Compressive strength of calcium sulfate is greater than cancellous bone, although tensile strength is slightly inferior. Plaster of Paris provides no internal strength or support, and therefore should only be used to fill small bone defects or in association with a rigid internal fixation.

Current applications concern spine fusion, packing of benign tumors or cysts after curettage, and trauma.^[22,23] However, in open systems, such as spinal arthrodesis, calcium sulfate has failed to achieve an optimal fusion rate, mainly because of early absorption.^[24] Plaster of Paris is very inexpensive, can be prepared easily, and has an indefinite shelf life. Interestingly, it may also serve as a vehicle for the administration of several agents such as antimicrobials, antibacterials, or possibly osteoinductive agents.

2.2.2 Calcium Phosphate Ceramics—Calcium phosphate materials account for most of the ceramic-based bone graft substitutes currently available. Since the 1950s,^[25] extensive experimental and clinical studies have reported the filling of bone defects in periodontics, oral and maxillofacial surgery, neurosurgery and orthopedic surgery.

Calcium phosphate ceramics (CPCs) are characterized by their chemical composition, which is similar to that of the mineral phase of calcified tissue, namely calcium hydroxyapatite. It is possible to control the composition of the untreated product by adjusting the calcium-to-phosphate ratio (Ca/P). Hydroxyapatite and β -tricalcium phosphate (β -TCP) are the most

widely used, mainly in combination in the so-called biphasic calcium phosphate ceramic. Hydroxyapatite can also be obtained from natural reef-building coral skeleton.^[26]

Porous CPCs are osteoconductive, biocompatible, and bioactive.^[27–28] For a given chemical composition, ceramics with lower Ca/P ratios, such as β -TCP, resorb more rapidly.^[29] The main drawback is an unpredictable biodegradation profile, and, subsequently, an undesirable loss of strength. The combination of different calcium phosphate (CaP) compounds is therefore used as a strategy to control bioresorption rates of the ceramics. Macroporosity (pore diameters >100 μ m) and pore interconnectivity seem to be the most important parameters for adhesion, proliferation, and differentiation of osteoprogenitor cells as a prerequisite for bone ingrowth.^[27,30,31] Resorption of porous CPCs begins with the dissolution of ionic precursors in the extracellular environment, and is driven by giant cells and macrophages. The material is progressively replaced by new lamellar bone.

CPCs are brittle and weak under tension and shear, and resistant to compressive loads. The compressive and tensile strengths of β -TCP are very similar to those of cancellous bone, whereas dense ceramics such as hydroxyapatite can resist up to 100 MPa in compression and have a much higher modulus of elasticity than bone. Therefore, CPCs are not indicated in constrained or load-bearing areas, unless they are associated with an osteosynthesis.

Conditions for CPC osteointegration include close contact between synthetic graft and host, optimal primary stability, and a well vascularized environment. CPCs have been used in the reconstruction of acetabular defects at the time of revision hip arthroplasty with encouraging radiological and histological results.^[32–34] On the femoral side, subsidence of the stem commonly described with morselized impacted bone allograft technique^[13,35,36] led Nich and Sedel^[37] to evaluate the reconstruction of femoral cavitory defects using a macroporous CPC. Satisfying bone stock restoration and very limited rate of subsidence were obtained. CPCs are effective promoters of fusion in spine arthrodesis.^[38] Several studies have reported successful use of CPCs in proximal tibial open-wedge osteotomy in the treatment of medial compartment osteoarthritis of varus knee.^[39,40] Macroporous ceramics have been employed successfully to fill cancellous bone void following fracture^[41–43] or curettage of benign bone tumors.^[44–47] To overcome the problem of the brittleness of CPCs, without reducing the bone-bonding properties, researchers are developing hybrid composites of CaP and polysaccharide such as chitosan.^[48–50]

2.2.3 Calcium Phosphate Cements—Self-hardening CaP cements were introduced in the late 1980s,^[51] and received approval by the US FDA in 1996. Apatitic CaP cements, such as Norian[™] SRS (Synthes, PA, USA), are viscous and moldable, but may be difficult to inject. In contrast, brushite CaP cements can be initially very liquid and still set within a short period of time. The cement setting reaction determines its mechanical and biological properties.

After hardening, CaP cements are highly microporous, which implies that their specific surface area is high, and, subsequently, ionic exchanges with the extracellular environment are significant. CaP cements degrade layer by layer, which theoretically allows no bone ingrowth, as opposed to open macroporous CaP blocks. The biocompatibility of apatitic CaP

cements is excellent, and their biodegradation is much larger than that of hydroxyapatite, although incomplete and slow.^[52]

CaP cements are brittle and have relatively low bending/flexural strengths. Mechanical properties of CaP cements depend on their composition, with brushite cements being slightly weaker than apatitic CaP cements. CaP cements can only be used in combination with internal or external fixation or in low- or non-load-bearing applications. Similar to CPCs, improvement of the material and mechanical properties have been achieved by incorporating biocompatible and bioresorbable reinforcement additives such as Vicryl meshes^[53] or chitosan.^[54]

Mechanical properties of NorianTM SRS allow for rapid load bearing and/or provide good additional stabilization in unstable fractures of the distal radius,^[55] tibial plateau,^[56] and calcaneus.^[57,58] Potential adverse effects of apatitic cements such as soft-tissue reactions have been described.^[59] CaP cements can also be used as delivery systems for therapeutic peptides, antibacterials, anticancer drugs, anti-inflammatory drugs, or growth factors.^[60]

2.2.4 Bioactive Glasses and Ceramics—Bioactive glasses and ceramics constitute a group of synthetic silicate-based materials, characterized by their bioactivity and their unique bone-bonding properties.^[61] Degradation products of bioactive glasses are entirely metabolized by the body. Bioactive glasses are composed of silicate (SiO₂), sodium oxide (Na₂O), calcium oxide (CaO), and phosphorous pentoxide (P₂O₅). By varying the proportions of sodium oxide, calcium oxide and silicon dioxide, a range of forms can be produced, from soluble to non-resorbable.

Bioactivity is dependent on chemical composition. Compositions for most rapid bonding to bone tissue range from 45 to 52% in weight of SiO₂, such as for 45S5 Bioglass[®] (US Biomaterials Corp., FL, USA).^[61] Bone bonding occurs after a rapid sequence of chemical reactions on the surface of the implant after contact with body fluids. Bioactive glasses are able to stimulate the growth and maturation of osteoblasts,^[62,63] and to promote the expression and maintenance of the osteoblastic phenotype^[64,65] upon cell/material contact.

The development of apatite/wollastonite (A/W) bioactive glass-ceramic^[66] resulted in a consistent improvement of the component with regard to mechanical strength, toughness, stability, and bone bonding.^[67,68] Clinical success in spine surgery has been documented.^[69] Particulate glass materials were employed to restore bone loss resulting from periodontal disease in experimental settings.^[70] Difficulties associated with the use of bioactive glasses in biomedical applications are related to their brittle behavior and weak mechanical properties. The combination of biodegradable polymers with bioactive glasses has been proposed to produce products that display improved mechanical properties compared with conventional glasses.^[71]

2.2.5 Biomimetic Nanocomposites and Nanopolymers—Bone tissue may be viewed as a nanocomposite system with a complex hierarchical structure, mainly composed of type I collagen (the organic phase), and hydroxyapatite nanocrystals (the mineral phase). Cells naturally interact with nano-structured materials with a surface roughness of < 100 nm

in a physiological environment. Such roughness can be mimicked by polymers combined with hydroxyapatite through the use of nanophase materials, also called nanomaterials. Nanocomposites made of β -TCP as a matrix and hydroxyapatite nanofibers were used to produce porous scaffolds.^[72] Hence, nanomaterials exhibit mechanical and osteoconductive properties that are superior to their conventional counterparts. Osteoblast adhesion is facilitated by the adsorption and bioactivity of fibronectin and vitronectin on nanophase materials.^[73] The ability of the nanometer surface structure to control cell functions^[74,75] and to promote cell proliferation and osteogenic differentiation of human mesenchymal stem cells has been shown.^[75–77] Accordingly, it was reported that the use of a hydroxyapatite/collagen nanocomposite as a carrier for the delivery of recombinant human bone morphogenetic protein (BMP)-2 (rhBMP-2) was effective in promoting anterior fusion of the cervical spine in a dog model.^[78] Although promising, no clinical trials involving nanomaterials as bone substitute have been reported yet.

3. Osteoinductive and Osteopromotive Growth Factors: Biologics as Bone Repair Promoters

In the last few decades, growth factors that enhance musculoskeletal tissue regeneration have undergone extensive preclinical investigation.^[79] Currently, the most relevant growth factors for orthopedic applications are BMP-2 and -7, two members of the transforming growth factor beta (TGF β) super-family currently approved for clinical use in the US and Europe.^[80] The clinical product containing rhBMP-7 is Osigraft[®] (Stryker, MI, USA). The active substance is connected to a collagen matrix. Between 3.5 mg and 7 mg of Osigraft is recommended for nonunions 9 months after traumatic tibial fractures or for nonunions of the adult skeleton, when initial treatment with autologous bone grafting is unsuccessful. rhBMP-2 is the second clinical BMP that is currently available, marketed as InductOsTM[®] (Europe)/Infuse[®] (USA) [Medtronic, MN, USA]. The clinical indications include lumbar spinal fusions and as a supplement for the treatment of open tibial fractures that are stabilized by an intramedullary nail. BMPs also have limited indications for craniofacial bone defects. The recommended dose is 12 mg for a fracture; the maximum dose is 24 mg. The implantation has to be done on a collagen matrix.

An osteoinductive substance stimulates the osteogenic differentiation of precursor cells. In this context, BMP-2 and -7 have been proven to be effective as stimulatory agents for the treatment of critical-size segmental bone defects in animal models.^[81]

Only a few prospective, randomized, controlled trials investigating the potential for bone regeneration of BMP-2^[82–84] and BMP-7^[85–88] exist. In patients with open tibial fractures, BMP-2 accelerated wound and fracture healing and showed a reduced rate of secondary intervention and infection.^[83] However, no improved fracture healing was detected for patients with open tibial fractures when an absorbable collagen sponge with BMP-2 was used with a reamed intramedullary nail for fixation.^[84]

BMP-7, when implanted with a type I collagen carrier, showed comparable results to autologous bone grafting when used for the treatment of tibial nonunions.^[87] For critical

size fibular bone defects, BMP-7 had a healing bridging rate of 80% on a collagen 1 matrix compared with 0% for untreated cases.^[88]

In addition to facilitating healing of long bone fractures as previously discussed, BMPs are also used for anterior^[89] and transforaminal^[90] lumbar interbody fusion. A combination of allograft with BMP-2 has shown significantly higher fusion rates when compared with allografting alone and comparable fusion rates when compared with autografting.^[89]

However, there is only limited evidence that BMP is more effective than controls for acute tibial fracture healing in human studies.^[91] Furthermore, BMP-7, when used supportively during distal radius corrective osteotomies, led to decreased fracture healing as assessed by X-rays; BMP-7 also caused osteolysis around the osteotomy when compared with autologous bone grafting.^[86] Osteolysis was also reported after BMP-2 was used during transforaminal lumbar interbody fusion 1 year after operation, as assessed by CT scans.^[92] Supraphysiological doses of BMPs are used for the treatment of fractures, though there seems to be a limited therapeutic window as extremely high concentrations can lead to osteolysis.^[93]

Biologics that promote new bone formation are classified as osteopromotive. Osteopromotive growth factors include platelet-derived growth factor (PDGF),^[94] TGF β 1,^[95] insulin-like growth factor-1,^[96] vascular endothelial growth factor^[97] and fibroblast growth factor,^[98] all of which demonstrated clear osteopromotive potential in preclinical studies.

Despite a large body of preclinical evidence for the osteopromotive potential of platelet-rich plasma (PRP) and the availability of several commercial PRP isolation systems, it has not been FDA-approved for orthopedic applications at the current time.^[99] PRP can be easily derived from whole blood samples and contains platelets, white blood cells, fibrinogen and a variety of growth factors, notably PDGF- $\alpha\beta$, PDGF- $\beta\beta$, TGF β 1, and vascular endothelial growth factor (VEGF).^[100] In the field of orthopedic surgery, PRP has been reported to improve wound healing after total knee replacement,^[101] to enhance anterior lumbar interbody fusion,^[102] and to improve treatment of nonunions.^[103] However, a recent prospective randomized controlled trial detected no difference between PRP and a control group in anterior cruciate ligament healing.^[104] PRP is most frequently used, with good results, for conservative orthopedic treatment of knee pain^[105] and tennis elbow^[106,107] that is thought to be due to degenerative causes. Conflicting results exist for treatment of chronic achilles tendinopathy with PRP. While Gaweda et al.^[108] showed pain reduction, de Jonge et al.^[109] found no differences between the PRP and the control group in their Grade 1 level of evidence study. Currently, there are several PRP-separation systems available which can lead to wide variations in platelet and cell numbers as well as levels of growth factors.^[110] High thrombin concentration may negatively influence platelet activity.^[111] Overall, the level of evidence of studies demonstrating successful clinical use of PRP is low;^[112] further studies are needed to clarify the relevance of PRP for the treatment of orthopedic diseases.

One of the most challenging issues is the controlled long-term delivery of the growth factors to the site of injury. The agents currently in clinical use for this purpose are BMPs on

carrier-beds of collagen^[84] or biodegradable polymers.^[113] These have drawbacks regarding an insufficient time span for growth factor release and a lack of consistency in the amount released over time.^[114,115] Further optimization by controlling growth release kinetics underlies the basis for the development of highly effective biodrugs in the field of musculoskeletal tissue regeneration. One example is a 'layer-by-layer' technique using polyelectrolyte multilayer entrapment of growth factors.^[116] An interesting tool to potentially improve the growth of bone adjacent to orthopedic implants and to decrease the rate of failure would be to coat the implant surfaces with growth factors.^[117]

4. Cell-Based Concepts for Bone Regeneration

Cells are key players in bone regeneration. Osteoblasts produce osteoid, an extracellular protein-based matrix, which mineralizes to become bone. Osteoprogenitor cells or mesenchymal stromal cells (MSCs) can differentiate into osteoblasts and induce bone formation. MSCs can be readily isolated from bone marrow aspirates and expanded in culture, and provide an excellent source of osteoprogenitor cells because of their *ex vivo* differentiation and proliferation capacity.^[118] Recently, the osteoinductive properties of MSCs have been shown in numerous preclinical studies.^[119–123] Human clinical trials employing MSCs are currently examining their potential for orthopedic applications besides their role in the treatment of hematologic, cardiovascular, and neurodegenerative disorders.^[124,125]

Cells can be delivered locally^[126–129] or systemically.^[130,131] Osteogenic cells are usually isolated from bone marrow aspirates and subsequently expanded *in vitro*. Bone marrow aspiration is minimally invasive with negligible morbidity.^[132] Currently, two ways exist to prepare cells for clinical application, each with their advantages and disadvantages. One method is to concentrate cells immediately in the operating room and place them directly on the site of injury. Unfortunately, only low cell numbers can be generated by this process. Another method is to expand the cells *in vitro*. By this method a very large number of cells can be generated. The two-step method has two disadvantages: one additional exposure to anesthesia is required for re-implantation of the cells, and there is a risk of an *ex vivo* cell dedifferentiation and infection during the cell expansion.

Autologous chondrocyte implantation was first described in a clinical setting by Brittberg et al.^[133] in 1994. It is a two-stage procedure used to primarily treat circumscribed chondral defects in the knee joint of young patients. The first stage consists of harvesting healthy chondrocytes by arthroscopy from a non-load-bearing area. The cells are then culture expanded *in vitro* for 3–4 weeks.^[134] The second stage of the procedure entails implanting the autologous cells into the defect area. While some of the first prospective and randomized studies have shown promising results for medium- and large-sized chondral defects,^[135–137] others detected no difference in the outcome, compared with the microfracture technique.^[138,139]

Another biologic in current clinical use is concentrated bone marrow aspirate. This contains a high number of MSCs and growth factors. It has been reported to improve the outcome of non-traumatic osteonecrosis of the femoral head when the bone marrow concentrate was

injected into the operative core compression site.^[140,141] Autologous bone marrow is also currently used to enrich bone allografts for revision arthroplasty of the hip due to its osteogenic properties.^[142] The application of bone marrow concentrate to the site of a lesion appears to be a relatively simple and safe method to improve bone healing.

Many patients and orthopedic surgeons wish to have a completely biological joint replacement without artificial materials such as metal, ceramic, and polyethylene implants. There has been no clinical case of biological total joint replacement therapy yet, though it is an actively investigated area of basic research. Recently, the successful regeneration of an entire rabbit humeral condyle was reported.^[143] This was accomplished by implantation of a customized anatomically shaped bioscaffold that was infused with collagen gel containing TGF β 3 to stimulate the chondrogenic differentiation of cells from the surrounding tissues. Advancements in this field of research will be of great interest in the next few decades.

5. Combination Products

Demineralized bone matrix (DBM) is the only allograft material that has osteoinductive capacity. In the 1960s, Marshall Urist discovered that DBM had the capability of inducing bone formation in an ectopic site.^[144–146] Several years later, the agent responsible for this was found to be a group of proteins and was appropriately named BMP.

To prepare allograft bone for clinical use, it must be frozen, freeze-dried, or decalcified.^[147] A decalcified bone graft is less antigenic than a simply frozen graft. This affords DBM a high osteoinductive capacity.^[147] An advantage of DBM is that it can provide osteogenic growth factors, e.g. BMPs, and it also serves as a structural matrix consisting primarily of type I collagen. Because of its high concentration of BMPs, DBM exhibits rapid mineralization of tissue,^[148] and high rates of connectivity to host bone.^[149] In order to have a bone graft with both structural stability and osteoinductive potential, combinations of structural cortical bone graft and DBM are often used clinically.

Potential indications for the clinical use of DBM include spinal fusion, healing of unicameral bone cysts, treatment of long bone lesions, management of nonunions, and acetabular revision surgery.^[150]

Various clinical studies compared DBM to iliac crest auto-grafting for spinal fusion^[151–153] and diaphyseal non-united fractures of the humerus^[154] and found similar healing results for autologous bone grafting compared with autologous bone grafting extended by DBM or DBM alone. For critical size fibular bone defects, a healing bridging rate of 60% was shown for DBM matrix compared with 0% for untreated controls.^[88] Some drawbacks also exist for DBM. There is a wide variability in the osteoinductivity of different DBMs.^[155] This is due to differing processing and sterilization methods that may reduce the amount of functional BMPs.^[156] Just as there is donor-dependent variability in the osteoinductivity of DBMs, there is also variability in the osteoinductivity of allograft bone^[157] and a potential risk for viral transmission.^[158] The clinical use of DBM is promising, but at this time there are only a few prospective randomized studies in existence. Long-term follow-up and outcome data are still needed to establish DBM as a reliable method for regular clinical use.

6. Hormone and Gene Therapy

Gene therapy is an interesting tool used to accomplish the local delivery of beneficial growth factors for bone regeneration.

Genetic modification of cells can have advantages compared with the simple supplementation of cytokines or growth factors.^[159] First, the selected proteins have a short half-life. Second, a single administration is usually not sufficient for a biological effect. Third, the costs for the required quantities of protein would be prohibitively high. Fourth, continuous protein synthesis by genetically modified cells increases the likelihood for the desired effect. Genetically modified autologous MSCs, (over)expressing osteogenic growth factors or cytokines, provide both autocrine and paracrine stimuli to induce and maintain osteogenic differentiation and are therefore promising cellular components for protocols aimed at site-specific bone repair.^[160] In addition, the systemic or intraosseous marrow re-implantation of autologous MSCs genetically 'corrected' for any skeletal degeneration-causing mutation, could help to solve problems of limited availability and suboptimal engraftment of allogeneic MSCs.^[161]

There are both viral and non-viral methods to accomplish the above, with the viral methods showing a higher transfer efficiency of target genes.^[162] Currently, due to safety reasons, only animal models exist to evaluate gene therapy for fracture healing. An interesting method being developed for future clinical use is the *ex vivo* adenoviral transduction of tissue grafts to continuously deliver growth factors such as BMP-2 over a limited period needed for fracture healing.^[162]

In addition to local agents, the systemic use of hormone therapy in fracture healing is under investigation. Growth hormone appears to have a positive influence on fracture healing in animals and humans.^[163] Parathyroid hormone (PTH) has been shown to have a positive effect on fracture healing, especially for osteoporotic bones.^[164,165] The latest large animal investigations have reported improved bone defect healing by local delivery of PTH.^[166] In addition to binding PTH to fibrin,^[166] incorporating PTH to biomimetic CaP coating^[167] also offers a potential option for future therapies in humans. At this time, hormone therapy for human fracture healing is only under off-label use as further investigation into the appropriate dosages and safety factors are still necessary.

7. Conclusions and Perspectives

Interest in the use of biologics in orthopedic surgery is rapidly increasing. Bone auto- and allograft techniques have been established for decades, however new methods such as the use of recombinant human BMPs or autologous chondrocyte implantation have only reached the status of being clinical procedures in the last few years. The local application of stem cells is currently only being performed at highly specialized centers. In the past 40 years there have been no large developments regarding orthopedic surgical techniques, but as Sir John Charnley stated, there have to be other developments to improve orthopedic surgery.^[168] Orthopedic biologics appear to have the best chance of improving the field in the years to come.

7.1 Recommendation

Orthopedic surgeons must recognize that their field is changing to encompass more prominent opportunities for modulation of biologic processes to enhance repair and reconstruction of musculoskeletal tissues. This knowledge base will continue to expand, as new strategies and techniques to facilitate musculoskeletal health evolve.

Acknowledgments

Supported in part by the Ellenburg Chair in Surgery, Stanford University. SBG holds stock/stock options from Biomimetica, Biomimetic Therapeutics, StemCor, Accelalox and Tibion.

References

1. Khan SN, Cammisa FP Jr, Sandhu HS, et al. The biology of bone grafting. *J Am Acad Orthop Surg*. 2005 Feb; 13(1):77–86. [PubMed: 15712985]
2. Kappe T, Cakir B, Mattes T, et al. Infections after bone allograft surgery: a prospective study by a hospital bone bank using frozen femoral heads from living donors. *Cell Tissue Bank*. 2010 Aug; 11(3):253–9. [PubMed: 19562516]
3. Goldberg, VM.; Akhavan, S. Biology of bone grafts. In: Lieberman, JR.; Friedlaender, GE., editors. *Bone regeneration and repair: biology and clinical applications*. Totowa (NJ): Humana Press; 2005. p. 57-65.
4. Ullmark G, Obrant KJ. Histology of impacted bone-graft incorporation. *J Arthroplasty*. 2002 Feb; 17(2):150–7. [PubMed: 11847612]
5. van der Donk S, Buma P, Slooff TJ, et al. Incorporation of morselized bone grafts: a study of 24 acetabular biopsy specimens. *Clin Orthop Relat Res*. 2002 Mar.(396):131–41. [PubMed: 11859236]
6. Hamer AJ, Suvana SK, Stockley I. Histologic evidence of cortical allograft bone incorporation in revision hip surgery. *J Arthroplasty*. 1997 Oct; 12(7):785–9. [PubMed: 9355008]
7. Hooten JP Jr, Engh CA, Heekin RD, et al. Structural bulk allografts in acetabular reconstruction: analysis of two grafts retrieved at post-mortem. *J Bone Joint Surg Br*. 1996 Mar; 78(2):270–5. [PubMed: 8666640]
8. Enneking WF, Mindell ER. Observations on massive retrieved human allografts. *J Bone Joint Surg Am*. 1991 Sep; 73(8):1123–42. [PubMed: 1890115]
9. Schreurs BW, Slooff TJ, Buma P, et al. Acetabular reconstruction with impacted morsellised cancellous bone graft and cement: a 10- to 15-year follow-up of 60 revision arthroplasties. *J Bone Joint Surg Br*. 1998 May; 80(3):391–5. [PubMed: 9619924]
10. Azuma T, Yasuda H, Okagaki K, et al. Compressed allograft chips for acetabular reconstruction in revision hip arthroplasty. *J Bone Joint Surg Br*. 1994 Sep; 76(5):740–4. [PubMed: 8083262]
11. Berry DJ, Müller ME. Revision arthroplasty using an anti-protrusio cage for massive acetabular bone deficiency. *J Bone Joint Surg Br*. 1992 Sep; 74(5):711–5. [PubMed: 1527119]
12. Gill TJ, Sledge JB, Müller ME. The Büsch-Schneider anti-protrusio cage in revision total hip arthroplasty: indications, principles and long-term results. *J Bone Joint Surg Br*. 1998 Nov; 80(6): 946–53. [PubMed: 9853483]
13. Gie GA, Linder L, Ling RS, et al. Impacted cancellous allografts and cement for revision total hip arthroplasty. *J Bone Joint Surg Br*. 1993 Jan; 75(1):14–21. [PubMed: 8421012]
14. Rogers BA, Sternheim A, Backstein D, et al. Proximal femoral allograft for major segmental femoral bone loss: a systematic literature review. *Adv Orthop*. 2011; 2011:257572. [PubMed: 22013538]
15. Martin WR, Sutherland CJ. Complications of proximal femoral allografts in revision total hip arthroplasty. *Clin Orthop Relat Res*. 1993 Oct.(295):161–7. [PubMed: 8403642]
16. Clatworthy MG, Ballance J, Brick GW, et al. The use of structural allograft for uncontained defects in revision total knee arthroplasty: a minimum five-year review. *J Bone Joint Surg Am*. 2001 Mar; 83-A(3):404–11. [PubMed: 11263645]

17. Engh GA, Ammeen DJ. Use of structural allograft in revision total knee arthroplasty in knees with severe tibial bone loss. *J Bone Joint Surg Am.* 2007 Dec; 89(12):2640–7. [PubMed: 18056496]
18. Backstein D, Safir O, Gross A. Management of bone loss: structural grafts in revision total knee arthroplasty. *Clin Orthop Relat Res.* 2006 May;446:104–12. [PubMed: 16672879]
19. Mnaymneh W, Emerson RH, Borja F, et al. Massive allografts in salvage revisions of failed total knee arthroplasties. *Clin Orthop Relat Res.* 1990 Nov;(260):144–53. [PubMed: 2225617]
20. An HS, Lynch K, Toth J. Prospective comparison of autograft vs. allograft for adult posterolateral lumbar spine fusion: differences among freeze-dried, frozen, and mixed grafts. *J Spinal Disord.* 1995 Apr; 8(2):131–5. [PubMed: 7606120]
21. Gibson S, McLeod I, Wardlaw D, et al. Allograft versus autograft in instrumented posterolateral lumbar spinal fusion: a randomized control trial. *Spine.* 2002 Aug; 27(15):1599–603. [PubMed: 12163718]
22. Gitelis S, Piasecki P, Turner T, et al. Use of a calcium sulfate-based bone graft substitute for benign bone lesions. *Orthopedics.* 2001 Feb; 24(2):162–6. [PubMed: 11284599]
23. Kelly CM, Wilkins RM, Gitelis S, et al. The use of a surgical grade calcium sulfate as a bone graft substitute: results of a multicenter trial. *Clin Orthop Relat Res.* 2001 Jan;(382):42–50. [PubMed: 11154003]
24. Niu C-C, Tsai T-T, Fu T-S, et al. A comparison of posterolateral lumbar fusion comparing autograft, autogenous laminectomy bone with bone marrow aspirate, and calcium sulphate with bone marrow aspirate: a prospective randomized study. *Spine.* 2009 Dec; 34(25):2715–9. [PubMed: 19940728]
25. Ray RD, Ward AA Jr. A preliminary report on studies of basic calcium phosphate in bone replacement. *Surg Forum.* 1951:429–34. [PubMed: 14931264]
26. Guillemin G, Patat JL, Fournie J, et al. The use of coral as a bone graft substitute. *J Biomed Mater Res.* 1987 May; 21(5):557–67. [PubMed: 2884221]
27. Egli PS, Müller W, Schenk RK. Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits: a comparative histomorphometric and histologic study of bony ingrowth and implant substitution. *Clin Orthop Relat Res.* 1988 Jul;(232):127–38. [PubMed: 2838207]
28. Holmes RE, Bucholz RW, Mooney V. Porous hydroxyapatite as a bone graft substitute in diaphyseal defects: a histometric study. *J Orthop Res.* 1987; 5(1):114–21. [PubMed: 3029358]
29. Le Huec JC, Clement D, Brouillaud B, et al. Evolution of the local calcium content around irradiated beta-tricalcium phosphate ceramic implants: in vivo study in the rabbit. *Biomaterials.* 1998 May; 19(7–9):733–8. [PubMed: 9663747]
30. Le Huec JC, Schaefferbeke T, Clement D, et al. Influence of porosity on the mechanical resistance of hydroxyapatite ceramics under compressive stress. *Biomaterials.* 1995 Jan; 16(2):113–8. [PubMed: 7734643]
31. Böhner M, Baumgart F. Theoretical model to determine the effects of geometrical factors on the resorption of calcium phosphate bone substitutes. *Biomaterials.* 2004 Aug; 25(17):3569–82. [PubMed: 15020131]
32. Oonishi H, Iwaki Y, Kin N, et al. Hydroxyapatite in revision of total hip replacements with massive acetabular defects: 4- to 10-year clinical results. *J Bone Joint Surg Br.* 1997 Jan; 79(1): 87–92. [PubMed: 9020452]
33. Oonishi H, Kadoya Y, Iwaki H, et al. Hydroxyapatite granules interposed at bone-cement interface in total hip replacements: histological study of retrieved specimens. *J Biomed Mater Res.* 2000; 53(2):174–80. [PubMed: 10713564]
34. Schwartz C, Bordei R. Biphasic phospho-calcium ceramics used as bone substitutes are efficient in the management of severe acetabular bone loss in revision total hip arthroplasties. *Eur J Orthop Surg Traumatol.* 2005 Jun; 15(3):191–6.
35. Kärrholm J, Hultmark P, Carlsson L, et al. Subsidence of a non-polished stem in revisions of the hip using impaction allograft. Evaluation with radiostereometry and dual-energy X-ray absorptiometry. *J Bone Joint Surg Br.* 1999 Jan; 81(1):135–42. [PubMed: 10068021]
36. Eldridge JD, Smith EJ, Hubble MJ, et al. Massive early subsidence following femoral impaction grafting. *J Arthroplasty.* 1997 Aug; 12(5):535–40. [PubMed: 9268793]

37. Nich C, Sedel L. Bone substitution in revision hip replacement. *Int Orthop*. 2006 Dec; 30(6):525–31. [PubMed: 16741734]
38. Passuti N, Daculsi G, Rogez JM, et al. Macroporous calcium phosphate ceramic performance in human spine fusion. *Clin Orthop Relat Res*. 1989 Nov.(248):169–76. [PubMed: 2805476]
39. Gaasbeek RDA, Toonen HG, van Heerwaarden RJ, et al. Mechanism of bone incorporation of beta-TCP bone substitute in open wedge tibial osteotomy in patients. *Biomaterials*. 2005 Nov; 26(33):6713–9. [PubMed: 15950278]
40. Hernigou P, Roussignol X, Flouzat-Lachaniette CH, et al. Opening wedge tibial osteotomy for large varus deformity with Ceraver resorbable beta tricalcium phosphate wedges. *Int Orthop*. 2010 Feb; 34(2):191–9. [PubMed: 19795122]
41. Schwartz C, Liss P, Jacquemaire B, et al. Biphasic synthetic bone substitute use in orthopaedic and trauma surgery: clinical, radiological and histological results. *J Mater Sci Mater Med*. 1999 Dec; 10(12):821–5. [PubMed: 15347959]
42. Jiang S-D, Jiang L-S, Dai L-Y. Surgical treatment of calcaneal fractures with use of beta-tricalcium phosphate ceramic grafting. *Foot Ankle Int*. 2008 Oct; 29(10):1015–9. [PubMed: 18851818]
43. Scheer JH, Adolfsson LE. Tricalcium phosphate bone substitute in corrective osteotomy of the distal radius. *Injury*. 2009 Mar; 40(3):262–7. [PubMed: 19246039]
44. Uchida A, Araki N, Shinto Y, et al. The use of calcium hydroxyapatite ceramic in bone tumour surgery. *J Bone Joint Surg Br*. 1990 Mar; 72(2):298–302. [PubMed: 2155908]
45. Yamamoto T, Onga T, Marui T, et al. Use of hydroxyapatite to fill cavities after excision of benign bone tumours: clinical results. *J Bone Joint Surg Br*. 2000 Nov; 82(8):1117–20. [PubMed: 11132269]
46. Galois L, Mainard D, Delagoutte JP. Beta-tricalcium phosphate ceramic as a bone substitute in orthopaedic surgery. *Int Orthop*. 2002; 26(2):109–15. [PubMed: 12078872]
47. Schindler OS, Cannon SR, Briggs TWR, et al. Composite ceramic bone graft substitute in the treatment of locally aggressive benign bone tumours. *J Orthop Surg*. 2008 Apr; 16(1):66–74.
48. Zhang Y, Zhang M. Synthesis and characterization of macroporous chitosan/calcium phosphate composite scaffolds for tissue engineering. *J Biomed Mater Res*. 2001 Jun 5; 55(3):304–12. [PubMed: 11255183]
49. Ding S-J. Preparation and properties of chitosan/calcium phosphate composites for bone repair. *Dent Mater J*. 2006 Dec; 25(4):706–12. [PubMed: 17338304]
50. Ito M, Hidaka Y, Nakajima M, et al. Effect of hydroxyapatite content on physical properties and connective tissue reactions to a chitosan-hydroxyapatite composite membrane. *J Biomed Mater Res*. 1999 Jun; 45(3):204–8. [PubMed: 10397977]
51. Brown, W.; Chow, LC. A new calcium phosphate, water-setting cement. In: Brown, PW., editor. *Cements Research Progress 1986*. Westerville, Ohio: American Ceramic Society; 1987. p. 352-79.
52. Frayssinet P, Rouquet N, Mathon D, et al. Histological integration of allogeneic cancellous bone tissue treated by supercritical CO2 implanted in sheep bones. *Biomaterials*. 1998 Dec; 19(24):2247–53. [PubMed: 9884037]
53. Xu HHK, Quinn JB, Takagi S, et al. Synergistic reinforcement of in situ hardening calcium phosphate composite scaffold for bone tissue engineering. *Biomaterials*. 2004 Mar; 25(6):1029–37. [PubMed: 14615168]
54. Xu HHK, Quinn JB, Takagi S, et al. Processing and properties of strong and non-rigid calcium phosphate cement. *J Dent Res*. 2002 Mar; 81(3):219–24. [PubMed: 11881631]
55. Sanchez-Sotelo J, Munuera L, Madero R. Treatment of fractures of the distal radius with a remodellable bone cement: a prospective, randomised study using Norian SRS. *J Bone Joint Surg Br*. 2000 Aug; 82(6):856–63. [PubMed: 10990311]
56. Lobenhoffer P, Gerich T, Witte F, et al. Use of an injectable calcium phosphate bone cement in the treatment of tibial plateau fractures: a prospective study of twenty-six cases with twenty-month mean follow-up. *J Orthop Trauma*. 2002 Mar; 16(3):143–9. [PubMed: 11880775]
57. Thordarson DB, Hedman TP, Yetkinler DN, et al. Superior compressive strength of a calcaneal fracture construct augmented with remodelable cancellous bone cement. *J Bone Joint Surg Am*. 1999 Feb; 81(2):239–46. [PubMed: 10073587]

58. Schildhauer TA, Bauer TW, Josten C, et al. Open reduction and augmentation of internal fixation with an injectable skeletal cement for the treatment of complex calcaneal fractures. *J Orthop Trauma*. 2000 Jul; 14(5):309–17. [PubMed: 10926236]
59. Welkerling H, Raith J, Kastner N, et al. Painful soft-tissue reaction to injectable Norian SRS calcium phosphate cement after curettage of enchondromas. *J Bone Joint Surg Br*. 2003 Mar; 85(2):238–9. [PubMed: 12678359]
60. Maus U, Andereya S, Gravius S, et al. BMP-2 incorporated in a tricalcium phosphate bone substitute enhances bone remodeling in sheep. *J Biomater Appl*. 2008 May; 22(6):559–76. [PubMed: 18194994]
61. Hench LL, Paschall HA. Direct chemical bond of bioactive glass-ceramic materials to bone and muscle. *J Biomed Mater Res*. 1973; 7(3):25–42. [PubMed: 4123968]
62. Xynos ID, Edgar AJ, Buttery LD, et al. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. *J Biomed Mater Res*. 2001 May; 55(2):151–7. [PubMed: 11255166]
63. Kaufmann EA, Ducheyne P, Shapiro IM. Effect of varying physical properties of porous, surface modified bioactive glass 45S5 on osteoblast proliferation and maturation. *J Biomed Mater Res*. 2000 Dec; 52(4):783–96. [PubMed: 11033562]
64. Bosetti M, Vernè E, Brovarone CV, et al. Fluoroapatite glass-ceramic coating on alumina: surface behavior with biological fluids. *J Biomed Mater Res A*. 2003 Sep; 66(3):615–21. [PubMed: 12918045]
65. Hattar S, Berdal A, Asselin A, et al. Behaviour of moderately differentiated osteoblast-like cells cultured in contact with bioactive glasses. *Eur Cell Mater*. 2002 Dec; 4:61–9. [PubMed: 14562247]
66. Nakamura T, Yamamuro T, Higashi S, et al. A new glass-ceramic for bone replacement: evaluation of its bonding to bone tissue. *J Biomed Mater Res*. 1985 Aug; 19(6):685–98. [PubMed: 3001094]
67. Kitsugi T, Yamamuro T, Nakamura T, et al. Bone bonding behavior of three kinds of apatite containing glass ceramics. *J Biomed Mater Res*. 1986 Dec; 20(9):1295–307. [PubMed: 3782183]
68. Ono K, Yamamuro T, Nakamura T, et al. Quantitative study on osteo-conduction of apatite-wollastonite containing glass ceramic granules, hydroxyapatite granules, and alumina granules. *Biomaterials*. 1990 May; 11(4):265–71. [PubMed: 2383622]
69. Kasai Y, Takegami K, Uchida A. Mixture ratios of local bone to artificial bone in lumbar posterolateral fusion. *J Spinal Disord Tech*. 2003 Feb; 16(1):31–7. [PubMed: 12571482]
70. Schepers E, de Clercq M, Ducheyne P, et al. Bioactive glass particulate material as a filler for bone lesions. *J Oral Rehabil*. 1991 Sep; 18(5):439–52. [PubMed: 1666125]
71. Oréfiçe R, West J, Latorre G, et al. Effect of long-term in vitro testing on the properties of bioactive glass-polysulfone composites. *Biomacromolecules*. 2010 Mar; 11(3):657–65. [PubMed: 20108891]
72. Ramay HRR, Zhang M. Biphasic calcium phosphate nanocomposite porous scaffolds for load-bearing bone tissue engineering. *Biomaterials*. 2004 Sep; 25(21):5171–80. [PubMed: 15109841]
73. Webster TJ, Ergun C, Doremus RH, et al. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. *J Biomed Mater Res*. 2000 Sep; 51(3):475–83. [PubMed: 10880091]
74. Dalby MJ, Riehle MO, Johnstone H, et al. In vitro reaction of endothelial cells to polymer demixed nanotopography. *Biomaterials*. 2002 Jul; 23(14):2945–54. [PubMed: 12069336]
75. Schneider OD, Loher S, Brunner TJ, et al. Cotton wool-like nanocomposite biomaterials prepared by electrospinning: in vitro bioactivity and osteogenic differentiation of human mesenchymal stem cells. *J Biomed Mater Res Part B Appl Biomater*. 2008 Feb; 84(2):350–62. [PubMed: 17618506]
76. Bernhardt A, Lode A, Boxberger S, et al. Mineralised collagen: an artificial, extracellular bone matrix—improves osteogenic differentiation of bone marrow stromal cells. *J Mater Sci Mater Med*. 2008 Jan; 19(1):269–75. [PubMed: 17597360]
77. Jie W, Hua H, Lan W, et al. Preliminary investigation of bioactivity of nano biocomposite. *J Mater Sci Mater Med*. 2007 Mar; 18(3):529–33. [PubMed: 17334705]
78. Itoh S, Kikuchi M, Koyama Y, et al. Development of a hydroxyapatite/collagen nanocomposite as a medical device. *Cell Transplant*. 2004; 13(4):451–61. [PubMed: 15468687]
79. Dimitriou R, Jones E, McGonagle D, et al. Bone regeneration: current concepts and future directions. *BMC Med*. 2011; 9:66. [PubMed: 21627784]

80. Bishop GB, Einhorn TA. Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery. *Int Orthop*. 2007 Dec; 31(6):721–7. [PubMed: 17668207]
81. Betz OB, Betz VM, Abdulazim A, et al. The repair of critical size bone defects using expedited, autologous BMP-2 gene activated fat implants. *Tissue Eng Part A*. 2010 Mar; 16(3):1093–101. [PubMed: 20035609]
82. Jones AL, Bucholz RW, Bosse MJ, et al. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects: a randomized, controlled trial. *J Bone Joint Surg Am*. 2006 Jul; 88(7):1431–41. [PubMed: 16818967]
83. Govender S, Csimma C, Genant HK, 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 Dec; 84-A(12):2123–34. [PubMed: 12473698]
84. Aro HT, Govender S, Patel AD, et al. Recombinant human bone morphogenetic protein-2: a randomized trial in open tibial fractures treated with reamed nail fixation. *J Bone Joint Surg Am*. 2011 May; 93(9):801–8. [PubMed: 21454742]
85. Calori GM, D'Avino M, Tagliabue L, et al. An ongoing research for evaluation of treatment with BMPs or AGFs in long bone non-union: protocol description and preliminary results. *Injury*. 2006 Sep; 37(Suppl 3):43–50.
86. Ekrol I, Hajducka C, Court-Brown C, et al. A comparison of RhBMP-7 (OP-1) and autogenous graft for metaphyseal defects after osteotomy of the distal radius. *Injury*. 2008 Sep; 39(Suppl 2): 73–82.
87. Friedlaender GE, Perry CR, Cole JD, et al. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am*. 2001; 83-A(Suppl 1)(Pt 2):151–8. [PubMed: 11712836]
88. Geesink RG, Hoefnagels NH, Bulstra SK, et al. Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. *J Bone Joint Surg Br*. 1999 Jul; 81(4):710–8. [PubMed: 10463751]
89. Slosar PJ, Josey R, Reynolds J. Accelerating lumbar fusions by combining rhBMP-2 with allograft bone: a prospective analysis of interbody fusion rates and clinical outcomes. *Spine J*. 2007 Jun; 7(3):301–7. [PubMed: 17482113]
90. Villavicencio AT, Burneikiene S, Nelson EL, et al. Safety of transforaminal lumbar interbody fusion and intervertebral recombinant human bone morphogenetic protein-2. *J Neurosurg Spine*. 2005 Dec; 3(6):436–43. [PubMed: 16381205]
91. Garrison KR, Shemilt I, Donell S, et al. Bone morphogenetic protein (BMP) for fracture healing in adults. *Cochrane Database Syst Rev*. 2010; (6):CD006950. [PubMed: 20556771]
92. Helgeson MD, Lehman RA Jr, Patzkowski JC, et al. Adjacent vertebral body osteolysis with bone morphogenetic protein use in transforaminal lumbar interbody fusion. *Spine J*. 2011 Jun; 11(6): 507–10. [PubMed: 21729801]
93. Baas J, Elmengaard B, Jensen TB, et al. The effect of pretreating morselized allograft bone with rhBMP-2 and/or pamidronate on the fixation of porous Ti and HA-coated implants. *Biomaterials*. 2008 Jul; 29(19):2915–22. [PubMed: 18407348]
94. Al-Zube L, Breitbart EA, O'Connor JP, et al. Recombinant human platelet-derived growth factor BB (rhPDGF-BB) and beta-tricalcium phosphate/collagen matrix enhance fracture healing in a diabetic rat model. *J Orthop Res*. 2009 Aug; 27(8):1074–81. [PubMed: 19170096]
95. Lee J-Y, Kim K-H, Shin S-Y, et al. Enhanced bone formation by transforming growth factor-beta 1-releasing collagen/chitosan microgranules. *J Biomed Mater Res A*. 2006 Mar; 76(3):530–9. [PubMed: 16331652]
96. Granero-Moltó F, Myers TJ, Weis JA, et al. Mesenchymal stem cells expressing insulin-like growth factor-I (MSCIGF) promote fracture healing and restore new bone formation in Irs1 knockout mice: analyses of MSCIGF autocrine and paracrine regenerative effects. *Stem Cells*. 2011 Oct; 29(10):1537–48. [PubMed: 21786367]
97. Beamer B, Hettrich C, Lane J. Vascular endothelial growth factor: an essential component of angiogenesis and fracture healing. *HSS J*. Epub 2009 Sep 9.

98. Willems WF, Larsen M, Giusti G, et al. Revascularization and bone remodeling of frozen allografts stimulated by intramedullary sustained delivery of FGF-2 and VEGF. *J Orthop Res*. 2011 Sep; 29(9):1431–6. [PubMed: 21445997]
99. Castillo TN, Pouliot MA, Kim HJ, et al. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med*. 2011 Feb; 39(2): 266–71. [PubMed: 21051428]
100. Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. *Acta Biomater*. 2011 Feb; 7(2):463–77. [PubMed: 20688199]
101. Berghoff WJ, Pietrzak WS, Rhodes RD. Platelet-rich plasma application during closure following total knee arthroplasty. *Orthopedics*. 2006 Jul; 29(7):590–8. [PubMed: 16866090]
102. Hartmann EK, Heintel T, Morrison RH, et al. Influence of platelet-rich plasma on the anterior fusion in spinal injuries: a qualitative and quantitative analysis using computer tomography. *Arch Orthop Trauma Surg*. 2010 Jul; 130(7):909–14. [PubMed: 19949805]
103. Sanchez M, Anitua E, Cugat R, et al. Nonunions treated with autologous preparation rich in growth factors. *J Orthop Trauma*. 2009 Jan; 23(1):52–9. [PubMed: 19104304]
104. Nin JRV, Gasque GM, Azcárate AV, et al. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy*. 2009 Nov; 25(11):1206–13. [PubMed: 19896041]
105. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2011 Apr; 19(4):528–35. [PubMed: 20740273]
106. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med*. 2006 Nov; 34(11):1774–8. [PubMed: 16735582]
107. Gosens T, Peerbooms JC, van Laar W, et al. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up. *Am J Sports Med*. 2011 Jun; 39(6):1200–8. [PubMed: 21422467]
108. Gaweda K, Tarczynska M, Krzyzanowski W. Treatment of Achilles tendinopathy with platelet-rich plasma. *Int J Sports Med*. 2010 Aug; 31(8):577–83. [PubMed: 20535661]
109. de Jonge S, de Vos RJ, Weir A, et al. One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial. *Am J Sports Med*. 2011 Aug; 39(8):1623–9. [PubMed: 21602565]
110. Mazzocca AD, McCarthy MBR, Chowanec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am*. 2012 Feb; 94(4):308–16. [PubMed: 22336969]
111. Han B, Woodell-May J, Ponticello M, et al. The effect of thrombin activation of platelet-rich plasma on demineralized bone matrix osteoinductivity. *J Bone Joint Surg Am*. 2009 Jun; 91(6): 1459–70. [PubMed: 19487525]
112. Yuan T, Guo S-C, Han P, et al. Applications of leukocyte- and platelet-rich plasma (L-PRP) in trauma surgery. *Curr Pharm Biotechnol*. Epub 2011 Jul 8.
113. Saito N, Murakami N, Takahashi J, et al. Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins. *Adv Drug Deliv Rev*. 2005 May; 57(7):1037–48. [PubMed: 15876402]
114. Kanematsu A, Yamamoto S, Ozeki M, et al. Collagenous matrices as release carriers of exogenous growth factors. *Biomaterials*. 2004 Aug; 25(18):4513–20. [PubMed: 15046942]
115. Nagahama K, Ueda Y, Ouchi T, et al. Biodegradable shape-memory polymers exhibiting sharp thermal transitions and controlled drug release. *Biomacromolecules*. 2009 Jul; 10(7):1789–94. [PubMed: 19425546]
116. Go DP, Gras SL, Mitra D, et al. Multilayered microspheres for the controlled release of growth factors in tissue engineering. *Biomacromolecules*. 2011 May; 12(5):1494–503. [PubMed: 21413682]
117. Wildemann B, Sander A, Schwabe P, et al. Short term in vivo biocompatibility testing of biodegradable poly(D,L-lactide): growth factor coating for orthopaedic implants. *Biomaterials*. 2005 Jun; 26(18):4035–40. [PubMed: 15626449]
118. Haynesworth SE, Goshima J, Goldberg VM, et al. Characterization of cells with osteogenic potential from human marrow. *Bone*. 1992; 13(1):81–8. [PubMed: 1581112]

119. Bruder SP, Kurth AA, Shea M, et al. Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. *J Orthop Res.* 1998 Mar; 16(2):155–62. [PubMed: 9621889]
120. Arinzeh TL, Peter SJ, Archambault MP, et al. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. *J Bone Joint Surg Am.* 2003 Oct; 85-A(10): 1927–35. [PubMed: 14563800]
121. Nishikawa M, Myoui A, Ohgushi H, et al. Bone tissue engineering using novel interconnected porous hydroxyapatite ceramics combined with marrow mesenchymal cells: quantitative and three-dimensional image analysis. *Cell Transplant.* 2004; 13(4):367–76. [PubMed: 15468678]
122. Kalia P, Blunn GW, Miller J, et al. Do autologous mesenchymal stem cells augment bone growth and contact to massive bone tumor implants? *Tissue Eng.* 2006 Jun; 12(6):1617–26. [PubMed: 16846357]
123. Korda M, Blunn G, Goodship A, et al. Use of mesenchymal stem cells to enhance bone formation around revision hip replacements. *J Orthop Res.* 2008 Jun; 26(6):880–5. [PubMed: 18271017]
124. Bernstein P, Bornhauser M, Gunther KP, et al. Bone tissue engineering in clinical application: assessment of the current situation. *Orthopade.* 2009 Nov; 38(11):1029–37. [PubMed: 19838667]
125. Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol.* 2007 Apr; 211(1):27–35. [PubMed: 17226788]
126. Jäger M, Jelinek EM, Wess KM, et al. Bone marrow concentrate: a novel strategy for bone defect treatment. *Curr Stem Cell Res Ther.* 2009 Jan; 4(1):34–43. [PubMed: 19149628]
127. Gan Y, Dai K, Zhang P, et al. The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion. *Biomaterials.* 2008 Oct; 29(29): 3973–82. [PubMed: 18639333]
128. Hernigou P, Beaujean F. Treatment of osteonecrosis with autologous bone marrow grafting. *Clin Orthop Relat Res.* 2002 Dec.(405):14–23. [PubMed: 12461352]
129. Wakitani S, Nawata M, Tensho K, et al. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med.* 2007 Feb; 1(1):74–9. [PubMed: 18038395]
130. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med.* 1999 Mar; 5(3):309–13. [PubMed: 10086387]
131. Horwitz EM, Gordon PL, Koo WKK, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci U S A.* 2002 Jun 25; 99(13):8932–7. [PubMed: 12084934]
132. Bain BJ. Morbidity associated with bone marrow aspiration and trephine biopsy-a review of UK data for 2004. *Haematologica.* 2006 Sep; 91(9):1293–4. [PubMed: 16956842]
133. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med.* 1994 Oct 6; 331(14):889–95. [PubMed: 8078550]
134. Strauss EJ, Fonseca LE, Shah MR, et al. Management of focal cartilage defects in the knee: is ACI the answer? *Bull NYU Hosp Jt Dis.* 2011; 69(1):63–72. [PubMed: 21332441]
135. Bentley G, Biant LC, Carrington RWJ, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br.* 2003 Mar; 85(2):223–30. [PubMed: 12678357]
136. Horas U, Pelinkovic D, Herr G, et al. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint: a prospective, comparative trial. *J Bone Joint Surg Am.* 2003 Feb; 85-A(2):185–92. [PubMed: 12571292]
137. Basad E, Ishaque B, Bachmann G, et al. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. *Knee Surg Sports Traumatol Arthrosc.* 2010 Apr; 18(4):519–27. [PubMed: 20062969]

138. Knutsen G, Drogset JO, Engebretsen L, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture: findings at five years. *J Bone Joint Surg Am.* 2007 Oct; 89(10):2105–12. [PubMed: 17908884]
139. Van Assche D, Staes F, Van Caspel D, et al. Autologous chondrocyte implantation versus microfracture for knee cartilage injury: a prospective randomized trial, with 2-year follow-up. *Knee Surg Sports Traumatol Arthrosc.* 2010 Apr; 18(4):486–95. [PubMed: 19820916]
140. Gangji V, De Maertelaer V, Hauzeur J-P. Autologous bone marrow cell implantation in the treatment of non-traumatic osteonecrosis of the femoral head: five year follow-up of a prospective controlled study. *Bone.* 2011 Nov; 49(5):1005–9. [PubMed: 21821156]
141. Gangji V, Hauzeur J-P. Treatment of osteonecrosis of the femoral head with implantation of autologous bone-marrow cells. Surgical technique. *J Bone Joint Surg Am.* 2005 Mar; 87(Suppl 1) (Pt 1):106–12. [PubMed: 15743852]
142. Ochs BG, Schmid U, Rieth J, et al. Acetabular bone reconstruction in revision arthroplasty: a comparison of freeze-dried, irradiated and chemically-treated allograft vitalised with autologous marrow versus frozen non-irradiated allograft. *J Bone Joint Surg Br.* 2008 Sep; 90(9):1164–71. [PubMed: 18757955]
143. Lee CH, Cook JL, Mendelson A, et al. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet.* 2010 Aug 7; 376(9739):440–8. [PubMed: 20692530]
144. Urist MR. Bone: formation by autoinduction. *Science.* 1965 Nov; 150(698):893–9. [PubMed: 5319761]
145. Urist MR, Dowell TA. Inductive substratum for osteogenesis in pellets of particulate bone matrix. *Clin Orthop Relat Res.* 1968 Dec; 61:61–78. [PubMed: 4236465]
146. Urist MR, Silverman BF, Büiring K, et al. The bone induction principle. *Clin Orthop Relat Res.* 1967 Aug; 53:243–83. [PubMed: 4870495]
147. Pacaccio DJ, Stern SF. Demineralized bone matrix: basic science and clinical applications. *Clin Podiatr Med Surg.* 2005 Oct; 22(4):599–606. [PubMed: 16213382]
148. Han B, Tang B, Nimni ME. Quantitative and sensitive in vitro assay for osteoinductive activity of demineralized bone matrix. *J Orthop Res.* 2003 Jul; 21(4):648–54. [PubMed: 12798064]
149. Ragni P, Lindholm TS. Interaction of allogeneic demineralized bone matrix and porous hydroxyapatite bioceramics in lumbar interbody fusion in rabbits. *Clin Orthop Relat Res.* 1991 Nov;(272):292–9. [PubMed: 1934747]
150. Drosos GI, Kazakos KI, Kouzoumpasis P, et al. Safety and efficacy of commercially available demineralised bone matrix preparations: a critical review of clinical studies. *Injury.* 2007 Sep; 38(Suppl 4):13–21.
151. Cammisia FP Jr, Lowery G, Garfin SR, et al. Two-year fusion rate equivalency between Grafton DBM gel and autograft in posterolateral spine fusion: a prospective controlled trial employing a side-by-side comparison in the same patient. *Spine.* 2004 Mar; 29(6):660–6. [PubMed: 15014276]
152. Sassard WR, Eidman DK, Gray PM, et al. Augmenting local bone with Grafton demineralized bone matrix for posterolateral lumbar spine fusion: avoiding second site autologous bone harvest. *Orthopedics.* 2000 Oct; 23(10):1059–65. [PubMed: 11045552]
153. Rang J, An H, Hilibrand A, et al. Grafton® & local bone has comparable outcomes to iliac crest bone in instrumented single level lumbar fusions. *Spine.* Epub 2011 Nov 8.
154. Hierholzer C, Sama D, Toro JB, et al. Plate fixation of ununited humeral shaft fractures: effect of type of bone graft on healing. *J Bone Joint Surg Am.* 2006 Jul; 88(7):1442–7. [PubMed: 16818968]
155. Wang JC, Alanay A, Mark D, et al. A comparison of commercially available demineralized bone matrix for spinal fusion. *Eur Spine J.* 2007 Aug; 16(8):1233–40. [PubMed: 17205237]
156. Ferreira SD, Dernel WS, Powers BE, et al. Effect of gas-plasma sterilization on the osteoinductive capacity of demineralized bone matrix. *Clin Orthop Relat Res.* 2001 Jul;(388): 233–9. [PubMed: 11451125]

157. Schwartz Z, Somers A, Mellonig JT, et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation is dependent on donor age but not gender. *J Periodontol.* 1998 Apr; 69(4):470–8. [PubMed: 9609378]
158. Kinney RC, Ziran BH, Hirshorn K, et al. Demineralized bone matrix for fracture healing: fact or fiction? *J Orthop Trauma.* 2010 Mar; 24(Suppl 1):S52–5. [PubMed: 20182237]
159. Partridge KA, Oreffo ROC. Gene delivery in bone tissue engineering: progress and prospects using viral and nonviral strategies. *Tissue Eng.* 2004 Feb; 10(1–2):295–307. [PubMed: 15009954]
160. Caplan AI. Mesenchymal stem cells and gene therapy. *Clin Orthop Relat Res.* 2000 Oct.(379 Suppl):67–70.
161. Pochampally RR, Horwitz EM, DiGirolamo CM, et al. Correction of a mineralization defect by overexpression of a wild-type cDNA for COL1A1 in marrow stromal cells (MSCs) from a patient with osteogenesis imperfecta: a strategy for rescuing mutations that produce dominant-negative protein defects. *Gene Ther.* 2005 Jul; 12(14):1119–25. [PubMed: 15815702]
162. Evans C. Gene therapy for the regeneration of bone. *Injury.* 2011 Jun; 42(6):599–604. [PubMed: 21489526]
163. Tran GT, Pagkalos J, Tsiridis E, et al. Growth hormone: does it have a therapeutic role in fracture healing? *Expert Opin Investig Drugs.* 2009 Jul; 18(7):887–911.
164. Peichl P, Holzer LA, Maier R, et al. Parathyroid hormone 1–84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. *J Bone Joint Surg Am.* 2011 Sep; 93(17):1583–7. [PubMed: 21915572]
165. Aspenberg P, Genant HK, Johansson T, et al. Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. *J Bone Miner Res.* 2010 Feb; 25(2):404–14. [PubMed: 19594305]
166. Arrighi I, Mark S, Alvisi M, et al. Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. *Biomaterials.* 2009 Mar; 30(9):1763–71. [PubMed: 19124152]
167. Yu X, Wei M. Preparation and evaluation of parathyroid hormone incorporated CaP coating via a biomimetic method. *J Biomed Mater Res Part B Appl Biomater.* 2011 May; 97(2):345–54. [PubMed: 21432993]
168. Wroblewski BM. Professor Sir John Charnley (1911–1982). *Rheumatology (Oxford).* 2002 Jul; 41(7):824–5. [PubMed: 12096235]

Fig. 1.

Biologics in orthopedic surgery. The field of orthopedic surgery is currently using and investigating many different biologics with an aim of improving cell and tissue regeneration. The current gold standard is the bone autograft. Additional agents presented here are osteoconductive scaffolds, growth factors, cells, combination products, hormone therapy, and gene therapy. The current status of each product, whether it is used in basic science investigations, undergoing clinical trials, or US FDA approved for clinical usage, is denoted by red, blue, and green, respectively. **BMP** = bone morphogenetic protein; **CPCs** = calcium phosphate ceramics; **DBM** = demineralized bone matrix; **MSCs** = mesenchymal stromal cells; **PRP** = platelet-rich plasma.