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Recommendations and Considerations for the Use of Biologics in Orthopedic Surgery

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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.

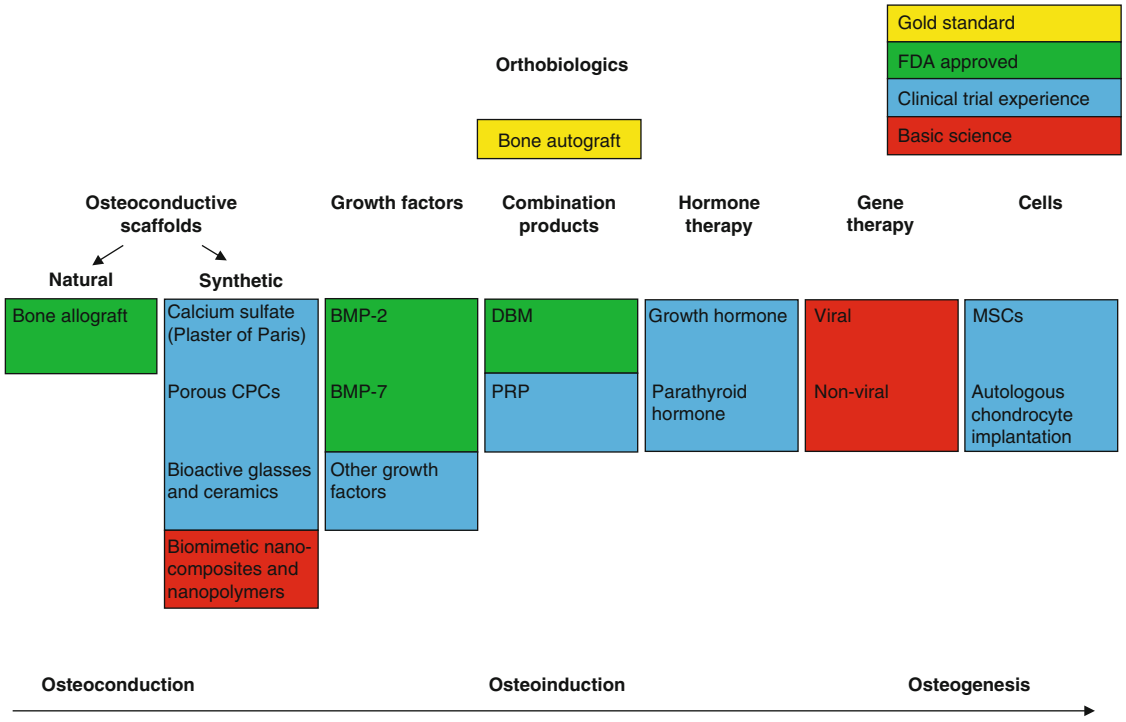

Fig. 1. Biologics in orthopedic surgery. The field of orthopedic surgery is currently using and investigating many different biologics with an aim of improving cell and tissue regeneration. The current gold standard is the bone autograft. Additional agents presented here are osteoconductive scaffolds, 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.

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 cavitary 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 bioadsorbable 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 μm) 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 cavitary 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 Norian™ 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 (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO), and phospohorous pentoxide (P_2O_5). 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 SiO_2, 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 (TGFβ) superfamily 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] TGFβ1,[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-αβ, PDGF-ββ, TGFβ1, 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 TGFβ3 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 autografting 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.

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