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The Use of Biomaterials in the Treatment of Fractures

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Dedico este tese aos meus pais e ao meu irmão pelo apoio incondicional e ao meu orientador Dr. João Torres pela total disponibilidade e apoio na sua elaboração.

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ABSTRACT

In the last decades, bone regeneration has acquired paramount importance in clinical practice associated with bone loss and deformities. Bone grafts are the second most used transplantation tissue worldwide. For a biomaterial to be considered ideal for bone replacement it should present four properties: osteointegration, osteoconduction, osteoinduction and osteogenesis. Autograft remains *the gold standard* in bone regeneration and repair demonstrating excellent osteogenic, osteoinductive and osteoconductive properties. Synthetic bone grafts must be biocompatible, structurally similar to bone, easy to use and with a good cost-effectiveness ratio. This review provides an overview of properties, clinical use, advantages and disadvantages of different biomaterials used in clinical practice.

Keywords

Bone Graft; Bone Substitute; Bone Repair; Bone Fracture; Orthopedics.

INTRODUCTION

Bone regeneration has acquired increasing importance in clinical practice associated with bone loss and deformities.(1) Bone grafts are the second most used transplantation tissue worldwide, generating values close to 2.5 billion dollars.(2-5) Around 2.2 million interventions using grafts are performed annually worldwide in the areas of orthopedics, neurosurgery and dentistry.(6, 7) The increase in research seen in this area can be explained not only by the lack of alternatives in clinical practice but also due to the growing need for tissue resulting from more aggressive surgical techniques in oncologic pathology, attempts to limbs rescue in severe trauma and reconstructive procedures.(8, 9)

For a biomaterial to be considered ideal for bone replacement, it should have different characteristics: a) osteointegration, which corresponds to the ability of chemically bond to the bone surface without the interposition of a layer of fibrous tissue;(10) b) osteoconduction, the capacity of bone ingrowth to support the construction of a structure for the migration of cellular elements (including osteoblasts, osteoclasts, mesenchymal stem cells and angiogenic cells);(10, 11) c) osteoinduction, the property of inducing proliferation or differentiation of stem cells to a osteoblastic phenotype;(12) and d) osteogenesis, bone formation from osteoblastic cells originating from the biomaterial itself or the host tissue.(11, 12) According to the Diamond Concept, in order to occur normal bone regeneration, some requirements must be fulfilled: the presence of osteogenic cells, growth factors, an osteoconductive scaffold and mechanical stability.(13)

All these requirements are only naturally present in autologous bone graft.(14) Synthetic bone grafts must be biocompatible, reabsorbable, structurally similar to bone (in terms of chemical composition, porosity and cell affinity), elicit minimal fibrotic reaction, provide support for osteogenesis, easy to use and with a good cost-effectiveness ratio.(15, 16) Ideally they should present a toughness and elasticity modulus similar to that of cortical tissue (with compressive strength between 90 and 230 MPa and tensile strength between 90 and 190 MPa) or trabecular bone (with compressive strength of 2-45 MPa they substitute.(14, 17, 18) This aspect is particularly relevant in locations where high loads (femur and tibia) or torsional forces (such as the humerus, radius and ulna) are present.(19) No bone substitute presents a biomechanical strength similar to that of cortical bone.(19) The epiphyseal/metaphyseal areas are characterized by their rigidity, high levels of vascularization and remodeling. Diaphysis are more elastic and formed by more dense and poorly irrigated bone, with slower remodeling processes.(1) The drawbacks encountered with some bone substitutes which exhibit these properties (calcium and aluminum) are a decreased or unpredictable resorption, handling difficulties (HA) and low clinical efficacy when foreign body reaction occurs (biodegradable polymer).(20-22) The implants can be integrated by mechanical contact or may promote bone growth by osteoconduction.(1)

Bone grafts, either of endogenous or exogenous origin, are often used to provide support, fill bone defects and improve skeletal deformities recovery.(23-25) The use of endogenous material presents certain limitations: the need for an additional surgical intervention, with frequent morbidity at the donor site and limited availability.(23-25) Limitations associated with the use of allograft include the possibility of disease transmission and immunogenic potential. Moreover, as the availability of allograft becomes insufficient, the need for use of synthetic bone substitutes, alone or in combination, will be higher.(26). Synthetic bone grafts are used primarily for its osteoconductive properties, but can also serve as a delivery system for local release of growth factors or antibiotics. Injectable forms allows the filling of cavities and improves screw fixation in osteoporotic bones. They may also allow earlier mobilization and loading by providing structural support in cases of articular impaction.(27, 28) One of the most promising application is the treatment of major deformities associated with fractures, with some studies reporting results with calcium phosphate and calcium sulfate equivalent or even superior to autograft.(29-33) Although most synthetic substitutes are more expensive than an allograft, they are sterile, easily stored and readily available for use.(34) Synthetic grafts carrying growth factors allow greater control of their release in time and space. The release of growth factors concomitant to resorption enables its continuous application in the area of bone growth, maximizing the power of the biomaterial and enhancing the orientation given by the scaffold.(26) The form of the scaffold serves as a substrate for bone growth and its geometry interferes with vascular leakage and resistance to invasion by fibrous-osseous tissue.(26)

Clinical validation of a bone substitute to one location does not imply a good applicability elsewhere.(15)

MATERIALS AND METHODS

The research was performed in MEDLINE through PubMed using the terms *"Biomaterials"*, *"Bone Graft"*, *"Bone Substitute*", *"Bone Fracture"* and *"Orthopedics"*. Articles focusing on biomaterials properties and clinical applications in Orthopedics were selected.

BODY OF WORK

1. Clinical Applications in Fractures

Multiple fractures benefit from the addition of bone graft or biomaterials during their surgical treatment (table 1), usually by ORIF (open reduction and internal fixation). As a general rule, we can say that these fractures occur in regions with a high percentage of cancellous bone, highly vascularized, with less resistance than the cortical bone. They are also regions, with the exception of the spine, adjacent to articular surfaces and, as such, the support provided by the graft or biomaterial used is of greater importance to prevent the loss of fracture reduction and consequent degenerative alterations which might ensue.

Bone Grafts
Autologous
Bone Marrow (BM)
Allogenic
Demineralized Bone Marrow (DBM)
Bone Substitutes
Hydroxyapatite
Tricalcium phosphate
Calcium phosphate cement
Bioactive glass
Calcium Sulfate
Aluminum Oxide
Coralline HA
Bone Grafts Based On Polymers
Collagen

Table 1 - Different approaches in the reconstruction of bone defects.

2. Bone Grafts

2.1. Autografts

Autografts are formed by osteogenic cells, bone matrix proteins and bone growth support tissue.(35) Autologous grafting is often harvested from the iliac crest as it allows easy access to trabecular bone, both in quantity and quality, the distal part of the radius and tibia may also be used.(15) It is *the gold standard* to fill bone defects, provide stable fixation, structural support and bone regeneration without complications due to its excellent osteogenic, osteoinductive and osteoconductive properties.(36, 37) Its clinical use includes nonunion in long bones and reconstruction of fractures with depresses tibial plateau.(33, 38, 39) They provide structural support to the implanted devices and become mechanically efficient as incorporation in the surrounding bone occurs.(40) Other advantages include its histocompatibility and minimal probability of immune rejection.(36) Its limited availability (more marked in pediatric patients) and viability, associated with significant morbidity (including blood loss, bruising, infection, fracture, loss of sensibility and chronic pain in the collection graft area) and increased surgery

time have limited its use.(11, 41-45) These complications can reach up to 25% of patients.(43, 46, 47) Direct proportionality between the extent of dissection performed in graft harvest and the severity of pain is observed.(48) Its application is also limited in elderly, patients with primary or secondary osteoporosis and in children with cancer.(49-52) Although there are no data on the quality of the autograft in the elderly, a lower mechanical strength and concentration of mesenchymal stem cells is predictable.(37) The use of autograft in the repair of large bone deformities presents rates of bone regeneration ranging from 60-100%.(53, 54) Failure rates variety may be associated with different harvesting techniques, handling and implantation of the graft, as well as incompatibilities with the patient.(54) The use of the Reamer-Irrigator-Aspirator technique (RIA) allows the irrigation of intramedullary canal and aspiration of marrow and bone fragments at the time of milling, allowing increases of 50 to 60 ml of collected graft volume with greater amount of growth factors.(52) However, there is a risk of iatrogenic fractures, decreased blood flow in the diaphysis and possible changes in erythropoiesis.(35, 55)

The autologous cancellous bone graft, the most commonly used form of autograft, has been considered to be more osteogenic than the cortical autograft because the spaces in its structure allow the diffusion of nutrients and revascularization.(45, 56-58) The trabecular graft is recommended to fill deformities or spaces but it does not provide considerable structural support. It plays a role mainly in osteoconduction because only osteoblasts and endosteal cells on the surface of the graft survive transplantation.(59) Its main advantage is the potential to transfer osteoprogenitor cells to the transplant area. Although the structural support is not immediate, trabecular bone graft ultimately acquires a similar strength to cortical graft between 6 and 12 months.(60) It is believed that the osteoinductive factors released from the graft during the resorption process and the cytokines produced during the inflammatory phase can contribute to bone regeneration.(59, 61)

The cortical autologous bone graft is essentially osteoconductive. Its dense and organized structure and good structural integrity is accompanied by reduced cellularity, limiting the osteogenic and osteoinductive potential.(45, 56, 62) The death of the majority of osteocytes after transplantation decreases even more the osteogenic potential.(62) Nevertheless, the surviving osteoblasts may exhibit osteogenic properties.(63, 64) Furthermore, their incorporation is slower when compared to cancellous graft.(45) The structural support is immediate with non-vascularized grafts, but the resorption and revascularization that ensues later weakens the graft in the initial 6 weeks comparing to vascularized graft.(63-65) The later have preserved osteoprogenitor cells and suffer a remodeling similar to the normal bone.(45) Some examples are: peroneal free grafts (peroneal artery), distal iliac crest graft (deep circumflex branches of iliac artery) and distal radial graft (intracompartmental arteries).(45) The cortical graft must be supported by an external or internal fixation in order to avoid fracture. They can be considered for segmental bone defects superior to 5-6 cm because they allow immediate structural support. Larger grafts need extended

period of resorption and graft fracture can occur if the osteogenesis doesn't run normally.(66, 67) The osteogenesis is influenced by the graft's implantation area, as the graft resorption and replacement occurs more rapidly at the ends of a long bone (composed by trabecular bone) than in the center (cortical bone).(66-70) It is vital that there is an intimate contact between the cortical bone graft and the surface on which it is placed. The bone-to-bone contact, together with low intensity pulsed ultrasound (LIPUS), are useful in the treatment of non-union and filling bone defects percutaneously.(71, 72) It is believed that this technique stimulates healing of the recent fracture and effectively promotes bone recovery in cases of delayed union or even in its absence, with a cure rate ranging from 70 to 93% in different non-randomized studies.(70)

2.2. Bone marrow

The bone marrow (BM) is used to stimulate bone formation by the action of cytokines and growth factors secreted from the transplanted cells.(73) Several clinical studies have demonstrated the efficacy of BM to treat bone deformities and nonunions.(74) Its can also be used as a graft expander in spinal arthrodesis.(75) The concentration of mesenchymal stem cells or the presence of endothelial osteoprogenitor cells, with their capacity to stimulate angiogenesis and restore blood flow in the fracture area, are some theories suggested for its effectiveness.(56, 76) Centrifugation allows separation of BM cells and plasma while preserving the osteogenic potential of the cells and decreasing injected material volume.(77) The addition of growth factors or collagen can further promote their proliferation and differentiation.(78) While some studies recommend centrifugation of the aspirate to increase cellular percentage, others report good results with its use as a graft expander (in cases of insufficient autograft) for experimental posterolateral spinal fusion.(77, 79) The combination of autologous BM with demineralized bone matrix (DBM) has been effectively used to fill bone defects as it is an excellent carrier due to its osteoinductive and osteoconductive properties.(73, 80) The injection of BM, with or without carrier, has been applied in the treatment of nonunions in various bones. This technique does not promote faster or greater extent of consolidation when compared to traditional bone grafting techniques.(81, 82) Its main advantage is that it can be performed percutaneously, almost without morbidity. The disadvantages are the lack of structural support and possible leakage of the material in liquid form.(45) Other more effective ways of releasing BM through semisolid matrices have been researched.(56) The necessary volume of BM to inject is not yet defined, but it is acknowledged that increasing of the aspirate volume is associated with the increase of positive alkaline phosphatase forming units. However, they are also more diluted.(83)

2.3. Allografts

Allografts correspond to bones obtained from cadavers or from patients undergoing total hip replacement, presenting osteoinductive and osteoconductive properties.(26, 84) They are the most widely used bone substitute and generally constitute the second option of the surgeon.(85) They are particularly important when there are large bone defects requiring good structural support or when there is an inadequate amount of autograft.(14) They may also be used to treat nonunions and in the increase of fracture repair.(19)

They are commercially available as powders, granules, cortical or trabecular fragments, wedges, blocks and strips.(19) Allografts of cortical or trabecular bone may be presented in a fresh form, a frozen form (which decreases the enzymatic degradation and immune response) or a lyophilized form (eliminates osteoinductive or osteogenic potential by removing water).(15, 86) The process also includes the sterilization with gamma radiation, radiation beam based on electron or ethylene oxide, further depleting it from its osteoinductive potential.(86) The fresh form is rarely applied due to increased risk of disease transmission and immune rejection.(87) The lyophilized allograft presents lower resistance and less osteoinductive properties when compared to frozen preparation.(15) Cortical allografts are often used to stabilize periprostetic hip fractures and in spinal surgery, where immediate load resistance is required.(45) The origin and processing of the allograft influences its properties.(37)

The allogenic grafts overcome some limitations associated with the use of autografts (readily available, without the need to sacrifice host structures or morbidity associated with the harvesting area).(37) Other advantages include its excellent osteoconductive potential and biomechanical properties.(19) However, they do not present autograft osteogenic potential due to the absence of viable cells.(88) The harvesting and preservation of these grafts are another limiting factor.(70, 89, 90) The irradiation leads to a reduction of bone graft osteointegration to 40%, as opposed to 80-100% usually observed.(91-97) Associations between allografts and transmission of infectious agents (e.g. HIV, HBV, HCV and other antibacterial agents), oncological, autoimmune diseases and toxins have been reported.(14, 15) Cases of HIV transmission are described (in frozen but not in lyophilized forms) and HCV.(98, 99) Nevertheless, this risk is significantly reduced by tissue processing and sterilization, with the disadvantage of affecting its biological and mechanical properties.(14, 100) The immune response and other biological properties tend to be reduced as a more aggressive processing of the allograft is used.(15) The presence of immune responses and difficulties in vascular infiltration slow its osteointegration and remodeling and, consequently, the new bone formation.(99) Other complications associated with their use include fractures and nonunions, with failure rates close to 15-20%.(14, 88, 101) Clinical results may vary considering that the graft quality is dependent on the donor.(19)

2.4. Demineralized Bone Matrix

Demineralized bone matrix (DBM), is produced by acid extraction of allograft.(86, 102) It has osteoconductive (type 1 collagen, noncollagenous proteins) and osteoinductive potential (BMPs, FGF, IGF, PDGF and TGF-β) and the trabecular collagenous structure of the original tissue.(86, 102-108) It is believed that its biological activity is due to proteins and growth factors in the extracellular matrix which become more bioavailable by the demineralization process.(11, 37) DBM is available in the form of gel, soft mass, granules, paste with bone chips and injectable paste.(15, 19) As it does not allow structural support, it is used primarily in a structurally stable environment.(11) It corresponds to a less immunogenic form of allograft.(109) It has been used effectively in the repair of high-risk fractures.(80, 106, 110-112) It can also be used in nonunions, fractures of the ankle or hindfoot and as an expander in spinal arthrodesis.(45, 113) Grafts with better results include DBM and BM composites in cases of unstable fixation and human DBM with calcium sulfate in calcaneus intraarticular fractures with split ends.(73, 80, 114) The revascularization process occurs rapidly, allowing it to function as a support for allograft. However, its clinical applicability is more significant as a graft expander and it can be used for the expansion of trabecular autograft when this is insufficient or the defect is too large.(115-118) There is a great variability in the amount of BMPs, as opposed to the approximately constant levels of growth factors.(115, 119) The storage, processing and sterilization can affect their osteoinductive properties.(11, 52) Accordingly, the DBM may be more efficient in osteoconduction in comparison to mineralized bone allograft.(118, 120) There are commercially available forms of DBM subjected to demineralization that doesn´t involve exposure to gamma radiation or ethylene oxide, reducing its influence on BMPs action.(60) It is not immunogenic because the antigenic surface of the bone is destroyed during demineralization and it has higher osteoinductive potential than the allografts.(76, 121) It can be combined with cortical and/or trabecular bone, promoting its osteoconductive properties.(56) Limitations to its use include the potential for infectious disease transmission (such as HIV), although there are no reported cases.(115) Different batches may possess different properties due to the large variability of donors used to harvest the graft.(11) The variation of BMPs concentration allied to reduced bioactivity further decreases its osteoinductive potential, already inferior to autograft.(122, 123) The potency of the different preparations depends on the manufacturing process.(86, 105) DBM is classified as a minimally handled material, thus there is less control over the manufacturers responsible for its processing.(52) In table 2 there is a summary of commercially available forms, clinical applications, as well as the advantages and disadvantages of different bone grafts.

Bone Grafts	Forms available	Clinical Applications	Advantages	Disadvantages
Autografts	Cortical, trabecular, vascularized and non-vascularized;	*Gold standard* for filling bone defects, providing stable fixation, structural support and bone regeneration;	Osteogenic, osteoinductive, and osteoconductive properties; Histocompatibility and diminished probability of immune rejection;	Limited availability and viability; Significant morbidity and increased operating period; Limited application in elderly, pediatric patients and primary or secondary osteoporosis;
BM		Skeletal deformities and nonunions; Graft expander in spinal fusion;	Performed percutaneously and almost without morbidity;	Absence of structural support; Leakage of material;
Allografts	Powders, granules, cortical or trabecular fragments, wedges, blocks and strips;	Treatment of nonunions; Applied to large bone defects requiring good structural support or when autograft is insufficient;	Osteinductive and osteoconductive properties; Biomechanical characteristics; Easily available and no morbidity associated;	No osteogenic potential; Transmission of infectious agents; Variable clinical results; Failure associated with fractures and nonunions;
DBM	Gel, malleable mass, strips, granule, paste with chips or injectable paste;	Primary use in structurally stable environment; Repair of high risk fractures; Graft expander;	Osteoconductive and osteoinductive potential; Less immunogenic allograft;	Support is not allowed; Different batches have different properties; Potency of different preparations depends on the manufacturing process;

Table 2 – Summary of commercially available forms, applications, advantages and disadvantages of different bone grafts.

3. Bone Substitutes

Ceramics are inorganic, solid and non-metallic materials which can be obtained by a process of heating followed by cooling (sintering).(19) It is a synthetic matrix of calcium phosphate that induces a biological response similar to bone.(1) They are available in blocks, wedges, granules, pastes and cements.(52) They allow adhesion, migration and proliferation of osteogenic cells, with new bone formation occurring in direct apposition to this biomaterial.(37) The resorption depends on the host, the type of material and the presence of osteoclasts and giant cells and it should preferably occur slowly.(37) The cell response to these biomaterials is influenced by its composition, rugosity, geometry, surface area and pore size.(52, 124) The pores are essential to new bone formation by allowing the migration and proliferation of osteoblasts, mesenchymal cells and vascularization. Additionally, they improve the interconnectivity between the implant and surrounding bone, allowing a greater mechanical stability.(125) Despite the porosity and pore size helping bone regeneration, it also reduces its mechanical resistance, limiting its use in loading areas.(17, 124) Hence, its application in areas with loading, shear forces, torsional or considerable bending is only possible with internal fixation.(75)

Synthetic forms of calcium phosphate may be divided into different groups according to their chemical properties, physical form and body reaction triggered.(126) There are three subgroups: hydroxyapatite (HA), tricalcium phosphate (TCP) and calcium phosphate cements (CPC).(126) Given the significant differences in the rate of resorption and porosity between TCP and HA, the mixture of these two different biomaterials becomes favorable - biphasic calcium phosphate (BCP).(26) An advantage of these ceramics is their good bone connection, allowing growth factors to intervene in bone regeneration despite the absence of osteoinductive properties.(26) Although they are recognized as osteoconductive, there are documented cases in which the coralline HA, TCP and CPC showed osteoinductive potential without

osteogenic factors addition. These osteoinductive properties seem to be due to its rugosity, topography, pore size and porosity which allow the accumulation of BMPs in circulation.(124, 127-129) The advantages of their use are: osteoconductive potential, bioactivity, biodegradability, long half-life, absence of toxicity, disease transmission and immunogenicity, unlimited availability in different shapes, porosities and compositions.(37, 52, 130) Handling difficulties, minimal structural support, lack of osteoinductive or osteogenic potential used alone are some disadvantages.(15, 37) The prolonged period required for its replacement by a newly formed bone and the inability to fill irregular defects makes them less useful if used alone in atrophic nonunions of long bones due to the lack of growth factors.(19, 86)) Its slow biodegradation, together with the compressive strength and the ability to osteointegration, make this class of biomaterials widely used by orthopedic surgeons.(45) One of the most promising aspects of calcium phosphate compounds is their potential use in local release of therapeutic or bioactive agents.(126)

3.1. Hydroxyapatite

The HA - C_{10} $(PO_4)_6$ $(OH)_2$ - is the main mineral component of the bone, contributing to its osteoconductive role and biocompatibility.(14, 131-133) It is available in ceramic or non-ceramic forms, as porous formulations, solid blocks or granules (table 3).(14) Its osteoconductive potential depends on pore size, porosity and biodegradation.(19) The resorption occurs for 2 to 5 years by dissolution or cell-mediated, depending on the manufacturing process, surface area and porosity.(134-139) This biomaterial can remain in the body for over 10 years.(139) In spite of not possessing osteoinductive potential, in the presence of HA, BM cells can differentiate and form a bone structure.(140)

It has been successfully used to coat metallic implants, improving their osteointegration.(141-143) It has obtained good results in greater bone defects.(144, 145) The granular form of HA has been applied alone or together with bone graft to fill bone deformities.(146) It can be modified or combined with other biomaterials in order to improve its absorption and function.(147) It has been suggested as an option for posterolateral lumbar spinal fusion to produce a fusion mass with high cell viability.(148, 149) Its use is associated with revision surgeries reduction, being possible to confirm radiographically the stability in the implant-bone interface and in the surrounding bone.(140) It may function as a carrier for growth factors and osteogenic cells, promoting its potential use as a bioactive delivery system.(150) The optimal pore size of a bioceramic must be similar to cancellous bone.(11) Pores less than 10 µm in diameter (microporosity) allow movement of body fluids and nutrients, as macroporosity (bigger than 50 µm) allows the colonization of bone cells.(151, 152) It has been reported that a pore diameter of 150-500 µm is optimal for bone growth.(153-155) Its higher density and crystal formation allow a higher mechanical strength and dissolution, favoring long term stability.(156) In contrast, an amorphous material with

greater porosity promotes bioactivity and bone ingrowth, but also suffers faster biodegradation, as seen with TCP.(156)

Synthetic HA has a good compressive strength but has little tensile strength.(14) It is a fragile biomaterial and prone to fracture.(14) The block form is difficult to shape, does not allow fibro-osseous growth and its elasticity modulus is much higher when compared to bone.(14) Despite the biocompatibility, its low solubility and mechanical properties differences from surrounding tissues and bone have limited its use.(157)

Formulations of HA doped with silicon and magnesium were recently created.(34) As silicon is involved in bone metabolism, a deficit in this compound can be associated with bone formation disturbances.(34) It can be used to fill bone spaces and in spinal arthrodesis with fixation.(145, 158) Magnesium ions promote bone resorption and formation by altering the structure of HA. Furthermore, it also improves the interaction with water, stimulating osteogenesis.(159) It can be used for filling spaces in spinal surgery, knee and trauma.(34) Despite being a biomaterial used for decades, there are few clinical studies evaluating the efficacy of HA.(34)

Pins coated with HA increase its fixation regardless of bone type and loading conditions. There is also a reduction in the rate of infection and enlargement during external fixation.(160, 161) Plasma coatings have been used on acetabular cups of hip prosthesis to fix and prevent complications, such as detachment associated with polymethylmethacrylate (PMMA).(162) Ceramic HA preparations are resistant to resorption *in vivo*, occurring at a rate of 1-2% per year.(10) Faster absorption kinetics occur on HA-coated with manganese and/ or zinc.(11)

3.2. Tricalcium Phosphate

TCP (Ca_3 (PO_4)$_2$) is a bioabsorbable and compatible material with chemical properties and crystallinity similar to the mineral phase of the bone.(11) It presents a stoichiometry similar to amorphous bone precursors, as opposed to HA which has similarities with mineral bone.(15) It is available in porous and solid forms, such as granules, blocks or wedges (table 3).(11, 14, 126) When adherent to healthy bone, osteoid production occurs directly in the ceramic contact surface, this osteoid mineralizes and, consequently, bone remodeling occurs.(15) It is used in its porous granular form, as less migration occurs when compared to solid granules due to early fixation by fibrovascular growth.(163) As the bone volume produced is less than the reabsorbed, it is used clinically as a complement to other less resorbable bone grafts or as an autograft expander.(14, 164) It can be used to fill small bone deformities of tibial, humeral, calcaneal and radial fractures and in spinal surgery.(19)) It can also be applied in intra-articular calcaneal fractures.(126, 165) Its possible use as a carrier for drugs has been studied.(166)

TCP is less brittle when compared to HA but it quickly loses mechanical strength due its faster resorption (1 year), which involves dissolution and osteoclastic resorption.(17, 139, 151) Its faster dissolution is an advantage compared to HA or CPC.(126) The porosity shows direct proportionality with degradation.(37) The compressive strength of the porous form of TCP is reduced to 30-40% at 4 months *in situ*, thus alerting to the need of protecting this biomaterial against loading until bone growth takes place.(167) During this period, the absence of loading and stabilization of the surrounding bone is necessary, unless it is applied in low mechanical *stress* areas or only subjected to compressive forces.(26) The porous form of TCP has areas of strength and compression similar to the trabecular bone.(156) As other preparations of calcium phosphate, this biomaterial proves to be fragile and brittle when subjected to pressure and shear forces, however, it is resistant to compressive forces.(168) No inflammatory responses were recorded, but there were foreign body reactions elicited by granules of material.(169, 170)

The hemyhidrate form of calcium phosphate ($CaSO_4$) has a rapid turnover, with most of the resorption taking place in weeks following the implantation.(26) It has the advantages of not inhibiting osteogenesis or aggravating pre-existing infections, low cost, accessible preparation and sterilization.(171) Its porous structure is highly variable, lacking in connectivity and requiring complete absorption along with bone growth.(26)

3.3. Calcium phosphate cement

It is an injectable paste of calcium which results from a combination of TCP, calcium carbonate and monocalcium phosphate, it has the highest compressive strength among osteoconductive bone substitutes, making it more useful for application in areas which require mechanical strength.(34, 172) As opposed to ceramics, it presents a solid structure with limited porosity.(19) It is available as a set of powders which are subsequently mixed to form a paste (table 4).(14) This radiopaque paste is injected into the bone defect under fluoroscopic control to confirm complete filling of the fracture area. At body temperature, it has a working time of 2 minutes, followed by a period of 8 minutes to stabilize.(14) The movement or manipulation during this period results in the crystallization process with fragmentation and early failure of the implant.(14) During the hardening process, in which an apatite type material is formed, the compressive strength is equal or even superior to that of the trabecular bone.(34) The resorption of this biomaterial is slow and it can be considered permanent.(34) The degradation of the cement depends on physicochemical properties and implantation area, and may take months, years or be incomplete.(17, 116, 173-175) Due to low resolution and limitations of conventional radiography, considerable bone remodeling has to take place before changes in the radiopacity are identifiable.(126) Radiography is, however, a useful technique to assess the position and containment of CPC after its implantation.(126)

Recent data indicate that the cements may directly initiate osteogenesis, with ionic exchanges possibly involved.(11, 176) During the process of dissolution and precipitation, the formation of a mineral layer of bone may induce bone formation by either mimicry of mineral bone structure or by the presence of osteogenic compounds (such as BMPs) in body fluids which can be concentrated on the newly formed mineral layer.(11) The compressive strength is similar to trabecular bone, but the resistance to shear forces and stress is considerably lower.(177) The pore size and resistance are fundamental in the usefulness of this biomaterial.(37) The combination of high biocompatibility, easy molding and the ability to set makes it useful in the repair of hard tissue defects.(178, 179) This cement has been used as an injectable biomaterial for bone replacement, as in vertebroplasty, kyphoplasty, distal radial fractures, calcaneanal fractures and femoral neck fractures and for strengthening screw fixation to vertebral pedicles (increasing the strength of the bone surrounding the implant).(126, 180-195) Using CPC with internal fixation in unstable trochanteric fractures appears to be useful to improve the fracture stabilization and reducing pain.(196) Clinical studies that applied this cement in tibial plate fractures have demonstrated safety and an equal or even superior outcome compared to autograft.(126, 197) The CPC allows filling the metaphyseal space created by this fracture, with improved stability and compressive strength, enabling earlier active joint movements and loading, without risk of fracture reduction.(34, 126, 198) Its application in impacted humeral fractures with open reduction and stabilization was associated with union of all fractures, with maintenance of reduction and no signs of osteonecrosis during follow-up.(199) Its application in intra-articular calcaneal fractures or depressed articular fractures has had good results.(193, 200) It is particularly recommended to fill subchondral defects in metaphyseal fractures primarily subjected to compressive forces, allowing structural support due its mechanical strength.(14, 126) The filling of the metaphysis with CPC in recent fractures or fractures with splitting tops after the initial treatment, in cases of comminuted fractures or significant bone loss has been studied.(191, 192) However, fixation is advised if located in areas subject to torsion, flexion or tension.(126) In order to overcome these limitations in mechanical properties, different types of reinforcing fibers have been studied.(201, 202) Another potential advantage of using these fibers is that if their absorption is faster than the cement and involves channels formation, which accelerate the resorption.(126) Other approach to stimulate bone resorption includes the incorporation of soluble particles in the cement or its combination with calcium sulfate.(203) It is used clinically as a substitute for PMMA, excluding osteoporotic burst type vertebral fractures.(194, 195, 204)

It has the advantage of not suffering dissolution or fragmentation when exposed to fluids and its hardening occurring without significant changes in temperature or pH.(14, 126) Moreover, the paste or injectable form is freely moldable and adaptable to bone defects.(11) Studies with cadavers reported less stabilization of distal radial fractures compared with Kirschner wires.(205) However, it allows early

mobilization and better clinical and radiological results.(14) Its use in distal radial fractures compared to external fixation showed a better clinical outcome at 7 weeks, but no differences after 3 months.(191) Since the stabilization occurs by isothermal reaction, different molecules can be added to improve its bioactivity. Different BMPs can be used to stimulate bone growth and repair of fractures, both *in vivo* and *in vitro*.(11) Antibiotics and chemotherapeutic agents may also be added without changing the mechanical strength of the cement.(14) One potential disadvantage is the extrusion to soft tissue, a complication that occurs frequently and is partially operator-dependent.(192) This possibility should be considered in the treatment of intra-articular fractures in order to prevent extrusion of the cement into the joint.(14) Regarding vertebroplasty or kyphoplasty, a cavity construction in the vertebral body, in theory reduces the risk of extrusion.(126) Another disadvantage is the need for close proximity to the host bone so that osteoconduction can be reached.(11) Even when reached, osteogenesis is often limited by the lack of osteoinductive properties.(11)

There are also non-ceramic forms of HA that are mixed during surgery, molded and set in vivo as porous HA.(14) Tetracalcium phosphate and dicalcium phosphate dihydrate are examples of such formulations that, when mixed with water, form a thick paste.(14) Stabilization occurs through an isothermal reaction and as the cement hardens it becomes a microporous form of HA.(14) During this period, the implant should not have contact with fluid as dissolution and stabilization in particulate form of the implant would occur.(14) Subsequently to the conversion to HA, it does not present risk of dissolution.(14) Non-ceramic form is more easily reabsorbed *in vivo*.(11) Other cements of HA include monohydrate of monocalcium phosphate, α-phosphate of tricalcium, calcium carbonate and a solution of sodium phosphate.(175)

3.4. Bioactive Glass

A form of bioactive glass, Bioglass®, is a compound based on silicon, biocompatible, osteoconductive and with the ability to bond directly to bone.(14, 206, 207) It is a solid inorganic compound consisting of sodium oxide (with solubility proportional to its content), calcium oxide, phosphorus pentoxide and silicon dioxide (the main component and responsible for bioactivity).(11, 14, 19, 208) It is formed at high temperatures and has a crystalline or amorphous structure.(45) It can be manufactured as beads, fibers or porous implants (Table 3).(19) This biomaterial is partially replaced by bone.(209, 210) It can be used to fill bone defects alone or in combination with allograft or autograft.(133, 146, 211-213) It has been effectively used as an graft expander and in metal implants coating to improve their osteointegration.(214) A strong mechanical bond between the bioactive glass and the bone is formed as a result of a gel layer rich in silica that is deposited on the glass surface when exposed to physiological aqueous solutions. In the gel, Ca^{2+} and PO_4^{3-} ions combine to form crystals of HA, similar to those of the

bone.(14, 208, 209) This bone-grafting connection allows the mechanical strength of the bioactive glass to be superior than calcium phosphate.(15) Its behaviour is influenced by its composition, pH and environmental temperature.(215-217)

The bioactive glass blocks are resistant to perforation and molding but may fracture during this process, thus being difficult to attach to the skeleton.(14) The use of a granular form in areas not subjected to loading to fill cavities does not provide benefits when compared to other hard pre-formed materials, except that its reabsorption can be faster than the HA particulate, with earlier recovery.(146) Its porosity allows the deposition of new bone after vascularization and osteoblast differentiation. This feature also plays a role in bone resorption and bioactivity.(218) In experimental studies in mice with trabecular bone deformities, bioactive glass had an excellent effect on the filling compared to autograft.(219) It was reported that the use of bioactive glass stimulated new bone formation by DBM. The absence of adverse cellular reactions proved its biocompatibility. The creation of microscopic roughness on its surface significantly improved bone bonding, with the intensity of this reaction affected by its composition.(220) Moreover, microroughness was associated with faster genes expression alteration involved in bone healing.(221) Histological studies *in vivo* reported moderate or absent inflammatory responses in the surrounding tissue and complete resorption of the graft.(222) In order to improve the fracture resistance stainless steel fibers may be incorporated.(223)

The glass ionomers are a glass powder of calcium/aluminum/fluorosilicate mixed with polycarboxylic acid/polyacrylic acid/polymaleic acid/citric acid, resulting in an exothermic reaction that produces a porous cement paste.(14, 15, 224) This paste hardens in few minutes, becoming insoluble to water.(14) Its elasticity modulus and compressive strength are similar to that of the cortical bone at the end of a day.(225, 226) This biomaterial has biocompatibility and osteointegrative properties similar to Bioglass®.(14) Its porous structure allows osteoconduction and thereby bone growth.(14) Glass ionomers are not replaced by bone as they are not resorbable and have been considered as an alternative to the PMMA cements, which use is limited by a significant exothermal reaction during polymerization.(14, 224) Furthermore, glass ionomers release proteins more efficiently than PMMA and are less likely to cause damage to heat labile proteins.(227) They may also be impregnated with antibiotics and other proteins for slow release.(15) One limitation is the difficulty to evaluate bone formation in radiopaque material.(75) Its use is contraindicated in contact with neural tissue or cerebrospinal fluid because the aluminum ions release is neurotoxic.(228)

3.5. Calcium Sulfate

Calcium sulfate has a crystalline structure and its action is presumably derived from an osteoconductive scaffold that allows blood vessels, osteogenic and fibrinogenic cells growth.(14) It has no osteoinductive or osteogenic properties and is relatively cheap (table 3).(45, 229) It is biocompatible, bioactive and resorbable by dissolution after 12 weeks.(14) While its reabsorption occurs through a chemical process that is independent of bone formation, the CPC and TCP are resorbed by cellular action (including osteoclastic cells), being the resorption dependent on bone formation.(126) It is commercially available as tablet, powder formulations and impregnated antibiotic formulations.(229) Given the speed of absorption, it is more used as graft expander than as structural or osteoconductive support.(105, 172, 229) It is generally weak and brittle, although there are changes with different formulations.(230) Depending on the heating method, it is possible to obtain an α or β hemyhidrate form, the former being more mechanically resistant by density differences.(230) A new formulation, *Osteoset-T*, has some advantages such as a homogeneous crystalline structure, a predictable rate of resorption and few trace elements. Thus, it allows a resorption rate similar to bone formation.(231) The calcium sulfate may be used to fill bone grafts cavities resulting from segmental defects and graft harvesting areas. In a randomized study by Kelly et al, *Osteoset-T* was used to fill contained and stable bone deformities after benign tumors, trauma or bone loss in the proximity of implants and as a supplement of fusion.(30) This study provided evidence for the use of calcium sulfate alone or as a graft expander in the those applications.(30) However, the variability of bone deformities and the absence of a true control group are some limitations in this study.(229) It can also be applied as a bone graft expander in spinal arthrodesis and for the treatment of proximal or distal tibial fractures and nonunions.(14, 68, 229, 232-236) Calcium sulfate has also been used in acute traumatic deformities and with DBM to fill trauma defects associated with calcaneal fractures.(235, 237) Although the rapid resorption leads to loss of some mechanical properties and excludes its use in loading areas, it may have advantages in infection because an impregnated antibiotic formulation can be used instead of gentamicin beads, thus avoiding a second surgery or a new graft to fill the deformity created by the cement.(11, 14, 229, 238) Calcium sulfate impregnated with tobramycin allows the reduction of infection and recovery period.(229) Different studies show that formulations of antibiotic-impregnated calcium sulfate allow an equal rate of infection eradication in comparison to antibiotic cement in chronic infections of long bones, requiring fewer surgeries in the future.(239-241) In its final form, the calcium sulfate has a higher compressive strength and a tensile strength slightly lower than the trabecular bone.(14) The implanted calcium sulfate needs to be adjacent to the viable periosteum or endosteum and it should stabilize in a dry environment as it can get soft and break when re-exposed to moisture.(14, 242) The lack of reliable mechanical properties *in vivo* limits its application to a limited area.(14)

Complications associated with its use include persistent serous drainage, superficial or deep infection and pseudoarthrosis.(229) Serous drainage is the most frequent complication and in most cases it is sterile and occurs until the biomaterial resorption is radiographically verified.(30, 229, 236, 239) Although in most cases drainage is treated with local procedures and does not require additional surgery, in a number of cases, the application of calcium sulphate with DBM was related to drainage in over 50% of the cases, with about a third of patients developing deep infection or requiring further surgery.(229, 243) Hence, using DBM and calcium sulfate is discouraged in the treatment of nonunion, particularly if there is a previous history of infection.(229)

3.6. Aluminum Oxide

Aluminum is a component of several bioactive materials and can also be used alone as bone graft.(14) Aluminum ceramic don´t undergo ionic exchange with the bone and subsequently they do not osteointegrate, with a mechanical connection to the bone resulting of pressures on the implant (table 3).(14). They have been applied as graft extenders and in wedge osteotomies.(14) The aluminum ceramics are hard and rigid and have superior resistance to HA in fractures associated with bending. However, its application in orthopedic surgery has been limited by its inability of osteointegration.(14)

Bone Substitutes	Forms Available	Clinical Applications	Advantages	Disadvantages
HA	Ceramic or non-ceramic forms as porous, solid formulations, blocks or granules;	Coating of metal implants; Filling of bone deformities, posterolateral lumbar arthrodesis and carrier for growth factors and osteogenic cells;	Good compressive strength; Possibility of modification or combination with other biomaterials to improve their resorption and function;	Low tensile strength; Fragile and prone to fracture; Low solubility; Block form is difficult to shape; Does not allow incorporation of antibiotics or growth promoting substances;
Tricalcium phosphate	Porous and solid forms, such as granules, blocks or wedges;	Complement to other bone grafts or as an autograft expander; Filling of small deformities in tibial, humeral, calcaneal or radial fractures and spinal surgery;	Tensile and compressive strength similar to trabecular bone; Less brittle than HA; Dissolved in 6 to 18 months;	Fragile and brittle when subjected to tension and shear forces; Rapid loss of mechanical strength; Does not allow incorporation of antibiotics or growth promoting substances;
Calcium phosphate cement	Set of powders;	Vertebroplasty, kyphoplasty; Distal radial fractures, fractures of the tibial plate, calcaneanal and femoral neck fractures; Strengthening of screws fixation to vertebral pedicles; Cavitational defects in metaphyses; Replacement of PMMA;	Higher compressive strength among osteoconductive substitutes; Stabilization in wet environment without dissolution or fragmentation; Moldable and adaptable to bone defects; Antibiotics and chemotherapeutic agents can be added;	Close proximity to host bone is needed; Extrusion to soft tissues; Resorption occurs during years;
Bioactive glass	Microspheres, fibers or porous implants;	Filling bone defects alone or in combination with autograft or allograft; Coating metal implants;	Ability to bind directly to bone; Strength significantly greater than calcium phosphate preparations;	Partially reabsorbed; Does not allow incorporation of antibiotics or growth promoting substances;
Calcium sulphate	Powders, blisters, impregnated antibiotic formulations;	More used as a graft expander (as in spinal arthrodesis, proximal or distal tibial fractures and nonunions); Filling spaces resulting from segmental defects, trauma and local graft harvest; Local release of antibiotics for infection prevention and treatment;	Biocompatible, bioactive and resorbable; Allows incorporation of antibiotics or growth enhancing substances;	No osteogenic, osteoinductive or structural properties in vivo; Use in loading areas excluded; Limited use to a restricted area; Complications include persistent serous drainage, superficial or deep infection and non-union;
Aluminum Oxide	Granules, blocks;	Expanders of grafts and wedge osteotomies;	Hard and rigid material; More resistant than HA implants;	Non-resorbable; Does not allow incorporation of antibiotics and growth promoting substances; Application in orthopedics limited by its inability of osseointegration;

Table 3 – Summary of commercially available, clinical applications, advantages and disadvantages of different bone substitutes.

3.7. Coralline HA

The coralline HA was developed to obtain an implant of HA with a consistent pore size and improved interconnectivity.(14) Substitutes based on coralline can be of natural origin or manufactured and they use the regular and highly permeable structure of marine coral (table 4).(14, 15) Coral calcium carbonate is processed to remove organic material, after being subjected to extreme pressure and heat in an aqueous solution of calcium phosphate.(14) This process allows the conversion of calcium coral carbonate entirely into calcium phosphate, as well as its sterilization.(14) A crystalline HA is obtained with pores size of 200-500 µm and with a structure similar to human trabecular bone.(11) The interconnectivity is vital because constrictions between pores limit vascular support for tissue ingrowth, leading to ischemia, which may play a role in implant failure.(244-246) The coralline HA is slightly higher in compressive strength than trabecular bone.(14) However, it is weak in tension, brittle and difficult to shape.(14) *Bucholz et al* reported a similar performance of autologous cancellous grafts and coralline HA in filling bone defects

resulting from the articular surface depression in tibial plate fractures.(15, 21) Both granules and blocks of coralline HA can be used to fill defects in the metaphysis after reduction of depressed articular segments.(68) It appears to be a clinically effective material that can be used in foot surgery, despite the slow resorption but without adverse effects.(247) This biomaterial has been successfully applied in lesions not subjected to loading, as the distal radial fractures.(248) However, this must be reinforced by internal fixation due to its initial mechanical weakness.(21) It may also be used as a graft expander in spinal arthrodesis.(249, 250) It was most recently used as a carrier for growth factors and BMPs.(11, 251-253) Such biomaterials can fill defects of about 8 cm with internal fixation so that failure in non-cyclic loading does not occur since they don't allow considerable resistance to stress.(21, 26) Initially it has no resistance nor the plastic properties of trabecular bone but as fibro-osseous tissue growth is completed, it becomes stronger but still less rigid than trabecular bone.(254) This aspect is advantageous for metaphyseal defects because it ensures structural support with good load distribution, reducing the likelihood of stress accumulation in the nearby articular cartilage.(255) Its use is contraindicated in articular surface defects due to the risk of migration of material to the joint.(11) Its best advantage is that the interporous structure allows the complete ingrowth of fibro-osseous tissue.(14) A considerable disadvantage is the mainly superficial delay in degradation which limits its application in places where bone remodeling is not as vital.(26)

3.8. Bone Graft Based Polymers

Bone graft based polymers vary in their physical, mechanical and chemical properties, and they may be natural or synthetic, degradable or non-degradable.(11) A commercially available form is a polymer based on natural products, composed by collagen fibers coated with HA and suitable for spinal arthrodesis (table 4).(256) Another injectable product based on resin has been used in areas subject to loading.(257) Polymer cements have been used to fill defects and in the reconstruction of complex fractures.(26) When fragmented, PMMA starts an osteolytic reaction, but as it is not resorbed, bone replacement does not occur in the area.(258) Other partially resorbable polymers that allow bone growth, such as poly (glycolic acid), have been added and applied successfully in fixing long bone fractures.(259, 260) The advantage of resorbable materials is the possibility of complete recovery of the deformity with no remaining foreign bodies.(11) They can also be applied as carriers of antibiotics or osteoinductive agents.(26)

3.9. Collagen

The collagen-based scaffolds are xenografts composed of bovine collagen combined with HA.(172) Collagen contributes to mineral deposition, vascular invasion and binding to growth factors, promoting

bone regeneration.(22) It is usually used as composite with other bone substitutes (HA and TCP with osteoconductive properties and BM with osteoinductive properties) and thereby it has shown inconsistent results, but its application as an expanding graft is effective.(table 4) As the compressive strength is lower than trabecular bone, they are often used for superficial defects correction.(172) Various studies have demonstrated the primary use of collagen as a carrier for osteogenic, osteoinductive or osteoconductive factors with mixed results.(120) The combination of collagen with autologous BM provides osteoprogenitor cells and growth factors, the combination with BMPs provides osteogenic precursors and HA greatly increases its incorporation.(11, 15) In a prospective randomized study, *Chapman et al* compared the autologous bone graft from iliac crest graft with collagen and calcium in the treatment of long bone fractures both with bone grafts and external or internal fixation.(261) There were no differences in the rate of union or functional measurements and they concluded that calcium and collagen graft with autologous BM can be used instead of autologous bone graft in patients suffering an acute traumatic long bone defect.(261) There are no studies proving that the autologous bone graft can be effectively replaced by collagen grafts for pseudarthrosis correction.(11) A composite of type 1 collagen and TCP (*Vitoss*) has been applied effectively in the spinal arthrodesis and elective surgery to the knee.(262-266) The collagen may be used with autologous BM in cases of long bone fractures with comminution or loss of cortical bone that requires bone graft when the internal and external fixation is planned.(68) It is not recommended to fill metaphyseal defects related to articular fractures due to the lack of structural support.(68) Its use is not indicated in the treatment of pseudarthrosis, except as an graft expander when the source of autologous bone graft is limited.(68) The collagen may have immunogenicity and it does not provide structural support.(15)

Bone Substitutes	Forms Available	Clinical Applications	Advantages	Disadvantages
Coralline HA	Granules or blocks, natural or manufactured;	Filling of bone defects resulting from articular surface depression in tibial plate fractures; Distal radial fractures; Graft expander in spinal arthrodesis; Carrier for growth factors;	Slightly higher in compressive strength than trabecular bone;	Weak in tension, brittle and difficult to shape; Limited application in areas where bone remodeling is not vital; Contraindicated in joint surface defects due to possible material migration;
Bone Grafts Based On Polymers	Natural or synthetic, degradable or non-degradable;	Spinal arthrodesis; Filling defects and reconstruction of complex fractures; Long bone fracture fixation and and in areas of loading;	Resorbable materials: possibility of full recovery without foreign bodies; Carriers for antibiotics or osteoinductive agents;	Some forms are not absorbable;
Collagen		Usually used with other bone substitutes; Graft expander; Carrier for osteogenic, osteoconductive and osteoinductive factors;	Contributes to minerals deposition, vascular invasion and binding to growth factors, favoring bone regeneration;	Compressive strength is lower than trabecular bone; It is not recommended to fill metaphyseal defects related to articular fractures; Not recommended in the treatment of nonunions; Can be immunogenic and does not provide structural support;

Table 4 – Summary of commercially available forms, clinical applications, advantages and disadvantages of coralline HA, bone grafts based on polymers and collagen.

4. Other Bone Substitutes and Future Perspective

In addition to the biomaterials already discussed, there is a variety of bone substitutes that have been developed.

Marine sponge skeletons and chitosan have proven to be effective biomaterials for tissue engineering.(267, 268) In addition, this compound has benefits in flexibility, strength, tension and elasticity modulus comparing to other biomaterials, allowing its molding without reducing its mechanical properties.(269)

The insulin-like growth factor (IGF-1), tissue growth factor (TGFβ), basic fibroblast growth factor (bFGF), a platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are examples of growth factors that can be used to stimulate bone formation.(270-276) Currently, only BMP-2 and BMP-7 were approved for clinical use.(86, 277) The BMP-2 is indicated for tibial open fractures and BMP-7 in the tibial non-union with prior failure or inability to perform graft.(52, 76, 278) Both can be used in spinal procedures - BMP-2 in anterior lumbar fusion and BMP-7 in revision surgeries following lumbar arthrodesis. (52, 76, 279)

Stem cells are an immature or undifferentiated cell that has the potential to create any daughter cell.(280, 281) There are two major sources of stem cells: somatic or adult and embryogenic.(280, 282-285) Despite being a promising option, they are associated with many ethical controversies related to human embryos destruction and their potential clinical application in regenerative therapies.(11) There are also difficulties in stem cells isolation and expansion.(11)

The plasma rich in platets (PRP) is an autologous platelet suspension subjected to centrifugation techniques.(45) Platelets have high concentrations of various growth factors (PDGF, VEGF and TGF-β) and, when activated by calcium chloride, they form a clot that is applied to the lesion.(45)

The parathyroid hormone (PTH) is an endocrine mediator involved in fosfocalcium metabolism and which action derives from interference in the balance between resorption and bone formation in favor of the latter.(45, 286) Their daily, pulsatile, low-dose administration has anabolic effects on bone.(45)

Gene therapy involves transferring genetic information into cells.(11) This technique uses viral proteins as a vehicle for the gene of BMPs, with continuous production of these proteins.(102) It was created to overcome limitations to the use of BMPs such as high cost and risk of local adverse effects.(130) Despite being a promising technique, more studies are required to prove its long-term safety, so that it can be used in clinical practice.(130)

CONCLUSION

Huge advances have been made in recent decades in the field of bone regeneration in the treatment of fractures. However, there is still no biomaterial able to gather all the advantages of the autograft without its drawbacks. The ideal would be a bone graft substitute that promotes new bone formation, with reduced morbidity and mortality, allowing early mobilization and cost-effectiveness. Despite different bone substitutes present known properties, a large variability in clinical response can be seen due to its marketing by different brands with different production methods. It is also necessary to have a greater regularization prior to its clinical approval, especially in bone substitutes that are considered as tissue and not implants because they do not involve considerable handling (such as DBM or BMPs). Although the future will likely focus on tissue engineering, it is crucial not to forget that its efficacy and safety have to be proven in humans before its approval for clinical use.

Concerning the treatment of fractures, it is important to understand that clinical outcomes depend on the combination of three components - patient characteristics (performance status, lifestyle, presence or absence of comorbidities), the fracture anatomical region (bone properties with their biological and mechanical needs) and the biomaterial characteristics. Thereby, in clinical practice an individualized approach to the patient is necessary.

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Anexos

Writing Papers for Biomaterials

Professor D.F.Williams, Editor-in-Chief
and
Peggy O'Donnell, Managing Editor

Introduction

Biomaterials is the leading journal that deals with biomaterials science and the related subjects of biocompatibility, medical devices, drug and gene delivery and tissue engineering. We are receiving manuscripts at 250 per month and publish over 7,000 pages per year. The high quality of the journal is beyond doubt. The maintenance, and hopefully even further improvement, of this quality is the concern and responsibility of authors, the editorial team, the referees and the publisher. The review process is central to the production of high quality published papers. The procedure, in general terms, is as follows. Manuscripts are received in the editorial office, via an online web submission and review system, and are read by the Editor-in-Chief. At this point the manuscript may be rejected because it does not match the scope of the journal. Only a few percent of manuscripts come into this category. The manuscript may also be rejected at this stage because, in the Editor's opinion, the quality of the paper is not sufficient to justify publication or because there would be very limited interest by the readership of the journal in the paper. This decision is never an easy one and the Editor takes into account the added value of the paper in comparison to other papers being published in ***Biomaterials*** in that specific area. Thus, a manuscript dealing with a slightly different way of sintering hydroxyapatite, or delivering a well-known drug in a slightly different manner, might be difficult to accept in view of the large number of papers on those subjects published recently. Approximately 35% of manuscripts are rejected on this basis, and the author is advised accordingly, usually within a couple of weeks and with a personalised letter of explanation.

If the Editor-in-Chief believes that the manuscript is of sufficient quality and interest to be peer reviewed, he will select appropriate referees from his database. The manuscript and abstract are sent by e-mail to referees who are invited to accept or decline the invitation within 10 days. If the referee agrees to conduct the review he is requested to complete his assessment and provide his report, in the EES web system, within three weeks. If a referee cannot review the paper, for any reason, the manuscript is sent to an alternate reviewer. This continues in cascade until the required number of reports is received. When the referee reports have been received, the Editor-in-Chief reads them and re-reads the manuscript. At this point he will either reject the paper, accept it without revision or request that the author revises the manuscript. A further 40% are rejected at this stage. Very few are accepted without revision. The authors normally receive copies of the referee's reports, although on occasion the Editor-in-Chief consolidates the referee's comments into his own report on the manuscript. If a revision is required, the authors are usually requested to complete this within a short, defined period of time, usually between 1 and 3 weeks. This time limit is specified to avoid the publication of work that becomes out of date. If revised manuscripts are received after the deadline, the editorial office may decide to have the paper re-refereed. It should be noted that only rarely will the Editor-in-Chief require that significant additional experimental work is required. If referees suggest that more work needs to be done in order to make the work publishable, the Editor-in-Chief will usually reject the paper, with a recommendation to the author.

It will be seen from the above summary that some 75-80% of manuscripts are rejected and 95% of those eventually accepted have to be revised. These are not exceptional figures for a high quality scientific journal. It is unlikely that the rejection rate will be lowered since it is

the intention to increase the quality of the journal, so that the acceptance criteria will be gradually raised. There are, however, many ways in which the overall quality of submitted manuscripts can be improved. This is important for several reasons. Too many manuscripts are received with obvious errors and poor quality presentation, which makes the editorial and review process more time consuming and difficult. It is not an easy task persuading referees to review papers and it is clear that they usually respond positively to well presented manuscripts but negatively (i.e. by refusing to do the review, or being late with the report) with poorly presented scripts. Obviously the shorter the editorial process the quicker will be the publication of the paper.

This present paper has been produced to give advice to authors on the presentation and submission of manuscripts to ***Biomaterials*** from the editorial perspective. It is not concerned with the logistics of submission, although a few aspects of this will be touched upon. It will cover manuscript content, style and length and will deal specifically about each part of a manuscript, from title to references.

Manuscript Content

Types of Manuscripts

Papers published in regular issues of ***Biomaterials*** are normally original research papers. Some Review papers are published but these are specially commissioned by the Editor-in-Chief. Leading Opinion Papers, which provide evidence-based scientific opinions on topical and important issues in biomaterials science, are also commissioned by the Editor-in-Chief. In both these cases, we do not accept unsolicited papers although the Editor would be happy to receive proposals for manuscripts in either category.

Scope

Because of the changing role of biomaterials in many areas of medical technology, the scope of the Journal is constantly evolving. The journal is relevant to all applications of biomaterials including implantable medical devices, tissue engineering and drug delivery systems. Indeed the journal is now divided into 9 sections, Biomaterials & Tissue Engineering, Biomaterials & Drug Delivery, Biomaterials & Medical Devices, Biomimetic & Natural Materials, Biocompatibility, The Materials Science of Biomaterials, Modelling of Biomaterial Performance, Biomaterials and Gene Transfer and Biomaterials for Biotechnology. Authors are requested on submission to specify the most appropriate section although the Editor-in-Chief may override the selection. It is very important that authors remain within the limits set out by these instructions. Thus, whilst we accept and indeed encourage manuscripts on drug delivery systems, the work must address materials science issues of these systems and not solely the pharmacology. Similarly, papers dealing with implantable devices must relate to the materials of those devices and not solely to clinical performance or biomechanics. Papers dealing with the synthesis and characterisation of new materials that might have potential as biomaterials cannot be accepted unless they are able to demonstrate some relevant biological performance data. Any manuscript that does not mention the materials actually used cannot be accepted and detailed information about the materials is normally required. It should also be noted that we rarely publish papers that only describe techniques, without any substantive new biomaterials science content.

Intellectual Property

Quite often, questions about proprietary names, trademarks or materials of an undisclosed specification, arise and great care has to be taken. It is acceptable for a material or a device to be described by a trade name as long as there is also a description of that material or device. However, we normally prefer that trade name not to be used in the title or in the list of key words. It is not acceptable for a paper to discuss a material that cannot be specified for confidentiality reasons. It should also be said that all authors have the responsibility of ensuring that they consider the intellectual property implications of manuscript submission.

Authors should be aware that the act of transmitting a manuscript to an editor, with the implicit assumption that the manuscript will be sent to referees, has already undertaken an act of disclosure which some legal jurisdictions may argue prevents a patent filing related to any aspect of the subject matter of the manuscript. Although in many jurisdictions of publication online is considered to be the date of disclosure we urge authors to take great care with the transmission of unprotected intellectual property. Also in the context of commercial aspects of biomaterials related products, we try to be very careful over the language used by authors to describe products, as they can be very misleading, often being written for 'marketing' purposes; papers that overtly promote a product, or denigrate competing products, are not acceptable.

Testing Results

A number of manuscripts have been received recently that report on tests carried out on biomaterials to determine biological safety, usually by compliance with the international standard ISO 10993. These manuscripts are normally rejected since this type of testing is done for regulatory purposes and is not scientifically based.

Splitting of Work

We have noticed recently that a number of authors are splitting pieces of work into very small packages and trying to publish these as series of papers. Whilst it is quite possible for a sequence of papers from one research group to be published, each paper has to be of sufficient significance to publish in its own right. It is unacceptable to submit a series of articles on the same subject matter, with duplication of much of the introduction, the methods, the discussion and references, and only small differences in the experimental work and results. Such submissions are usually returned with a request to consolidate them into one manuscript. It is also noticeable that a number of authors are submitting papers to ***Biomaterials*** that bear much similarity to papers submitted elsewhere, perhaps with sufficient differences to avoid any claim of publishing the same work twice, but only just. The editorial office is monitoring this situation and authors are asked to avoid this practice.

Incremental Work

There is one further feature about the manuscript content that should be emphasised. Quite often a manuscript is received that is technically and scientifically sound and fits the overall scope of the journal, but adds very little to our body of knowledge on the subject. Typically this happens when the data obtained and the conclusions drawn show only a minor incremental advance. When considering the publication times of journals and the overall level of interest generated in each paper, it is often difficult to justify the inclusion of such papers. Equally we do not usually accept papers that only provide data that supports or confirms existing knowledge.

Manuscript Style, Length and Structure

The guide to authors gives some sound advice about the structure and style of a manuscript. Authors should note that the following sequence is normally required: title, authors, affiliations, abstract, keywords, introduction, materials and methods, results, discussion, conclusions, acknowledgements, appendix (where necessary), figure captions and tables. Review papers may have a different format within the main text. Failure to follow these instructions leads to delays.

Language

Somewhere in the region of 75% of papers submitted are from authors who do not have English as their first language. The editorial team are sympathetic to these authors and try to help when there are difficulties, but it is in the best interests of authors to produce manuscripts

with high quality English in their first submission. The referees chosen for ***Biomaterials*** all understand the situation, and we in fact use many referees who do not have English as their first language, but it is inevitable that their view of a paper will be adversely affected if it is very difficult to read. In many cases we have to ask authors to have their manuscript checked and re-written by an English speaking person. Better use of spell-check and grammar-check software would also be helpful. It would be very beneficial if this could be done with the original submission rather than during the revision stage.

Length

There is no prescribed length of papers however the current average is 10 printed pages and we are seeking to reduce this to 8. The guide to authors urges them to write as concisely as possible. There are good reasons for this. Papers that are concise are more easily read by referees and by ultimate users of the journal. It also means that more papers can be published in each issue, thereby reducing publication times. It is important, of course, that the manuscript is sufficiently robust and substantive to convey accurately the significance of the work, but this can be achieved with careful attention to style of the text.

Title

The title is obviously the major factor that determines who will find and read the paper and great care should be taken with it. The title should be sufficiently informative so that the reader can immediately assess its likely relevance, but without being excessively long. The title does not have to convey the results or the conclusion, nor indeed does it have to specify the techniques. It is best to avoid sentences as titles; the best titles have between six and twelve words, with no verbs. As noted earlier, trade marks or proprietary names should be avoided.

Authorship

This is extremely important. In order to avoid later recriminations or even lawsuits, it is essential that all people who have played a significant role in the work and preparation of the manuscript are included in the list of authors and that, equally, there should be no authors who listed purely out of courtesy or local politics. Papers may be published by a single author or by a group of up to ten or twelve authors. Lead authors should be aware that papers lose some credibility if there are far more named authors than could have possibly been involved with the work in any significant way. It is important that authors follow the declaration of consent to submission as given in the guidelines.

Each paper should have a corresponding author. It does not matter where in the list of authors the corresponding author is placed, and it is recognised that different laboratories and institutions have different policies on this. However, the corresponding authors should, as the name implies, be the person who corresponds with the editorial office and who will be the lead correspondent with any reader who wishes to communicate with the authors once the paper has been published. Far too often the editorial office receives requests for information about a manuscript from authors who are not the corresponding author. The editorial office communicates only with the corresponding author. . The corresponding author must provide a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from biomaterials@online.be.

We would also like to standardise the way in which the author's names are quoted, but this is difficult because of cultural differences. We request the use of Christian name (given name), middle initial (if any) followed by surname (family name).

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Mandatory Author Declaration

An Author Declaration is a mandatory and integral part of a submission. This Declaration covers a number of logistic and ethical issues. A template for the covering letter is found on the ***Biomaterials*** website. Authors may save the template, obtain the required signatures and then upload it as a part of their submission. All authors need to physically sign the form. It cannot be emailed, faxed or sent by post.

Keywords

Keywords have become very important with respect to literature searches and many search engines operate through the listing of these words. It is in the author's interest to think carefully about the words that will attract interested readers to their paper. A list of preferred key words has been compiled by the Editor-in-Chief and may be found in the Guide for Authors. There is little point in using very generic terms such as biomaterial, implant, drug, tissue engineering and prosthesis as key words. Equally there is no point in using obscure names, and it is best to avoid the author's own abbreviations. As noted earlier, trade names should be avoided.

Abstract

Next to the title, the abstract will be the second most important point of entry to the paper since most search facilities will print the abstract as part of the service, and far more people will read the abstract than the full papers. The abstract should be concise and informative. It is not the place to expand on techniques or discuss philosophy, and the conclusions that it expresses have to be an accurate reflection on what was found. Abstracts should be not used to exaggerate the significance of the work and they should not contain subjective opinions on this importance or speculate how a material might be used. Very commonly submitted abstracts will include a phrase such as 'material X is very biocompatible and shows promise for use in orthopaedic implants'. This is rarely a sensible approach to writing an abstract. We do not require the abstract to be split into sections (e.g. background, experiments, results, conclusions) as demanded by some other journals. The instructions specify a length of 100 – 200 words. Most good abstracts are around 150 words in length, as a single paragraph.

Introduction

The Introduction, as the name implies, should introduce the background to the work that has been carried out, effectively providing the scientific rationale. It should contain sufficient citations to the key literature to support this rationale and should lead to a clearly stated hypothesis or set of objectives. Authors should assume that the readership of the journal is well-informed and there is no need for any generic educational background. For example, in a paper on wound healing it is not necessary to take the first page to explain the ideal characteristics of wound dressing materials, or in a paper on drug eluting stents it is not necessary to describe all of the competing technologies that address in-stent restenosis. The introduction should rarely be more than two manuscript pages long. It should not pre-empt the Results, Discussion or Conclusions.

Materials and Methods

This section should specify exactly what was done experimentally, with sufficient detail for the reader to be able to repeat the experiments if he wishes. It is acceptable to refer to other publications if the methods have been used elsewhere, for example the MTT test is used very widely and it is unnecessary to repeat the details unless there has been a departure from standard practice. It is not, however, acceptable to refer to the author's own work if it has been published in relatively inaccessible places, including PhD theses and non-English language journals. All of the experimental work discussed in the paper should be described in

this section. Materials used in the work should be described in appropriate detail, including sources of commercial supply or synthetic routes, and all major equipment should be specified with the manufacturers name, reference number and location. Animal experiments should be described in good detail but with sensitivity. Where institutional or regulatory rules apply to the conduct of the experiments they should be quoted. If any of the experimental work has been performed by a laboratory or organisation that is not represented in the list of authors, it should be explained here.

Results

Ideally the Results Section should be separate from the Discussion, but there is some flexibility here. The section should, obviously, be factual and it is best to avoid any philosophy or speculation. Authors should consider very carefully how to present their data. It should not be presented in multiple formats (i.e. the same data should not appear in figures and tables). If the data is displayed very effectively in either a table or figure, it should not be necessary to explain results in great detail in the text, but rather to use the text as a medium for emphasising the most significant data. It is occasionally acceptable not to provide actual evidence of the data, but this should not be done when the data is critical to interpretation, for example discussing crystallinity without showing XRD graphics.

Discussion

This section should summarise the nature of the observations and attempt to place this data into the context of the existing body of literature and, where appropriate, to express opinions about the significance of the work as far as biomaterials science is concerned. It should not be repetitive of the Introduction. It is entirely valid to suggest the potential implications of the work but without too much speculation. It is particularly important not to extend the discussion into areas that are not supported by the facts that are in evidence. Experiments that address the mutagenicity potential of implantable metals should not lead to discussions about the generic biocompatibility of these materials, for example.

It is also important that new data is not brought into evidence in the discussion. Several recent manuscripts have set out the experimental methods and results in the correct sections, but then the authors described quite briefly some additional experiments in the discussion and used those results to support their conclusions. This is not acceptable. Equally authors cannot cite their as-yet unpublished work to support the discussion.

Conclusions

Many authors end the Discussion section with a paragraph on the conclusions. This is not the best way to draw the manuscript to an end, and we require that conclusions be separated into a distinct section. This should not be too long, nor should it be repetitive of the discussion, and especially should not bring new ideas into the paper. The conclusions have to be based on the facts in evidence and should be limited to reasonable speculation about the significance of the work. The editorial team are particularly vigilant over the use of unjustified, exaggerated language in the Conclusions section.

Acknowledgements

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References

Instructions for the preparation of the list of references are given in the guidelines to authors; the designated form is modified Vancouver. These instructions should be followed exactly; failure to do this is one of the most common faults with manuscripts and causes frustration all round. Note that this system requires the names of all authors. Only where there are more than six authors can the abbreviation et al be used, after the name of the sixth author. There is no formal guidance on the number of references quoted, but in practice the best papers have between 20 and 30. It is better to avoid too many citations to the author's own work, and it is good to have a balance between the older seminal papers that lay the groundwork for that particular area and recent quality papers that have contributed serious input into the subject. Documents that have limited circulation, obscure journals or books, especially those out of date, and electronic sources (e.g.web-sites) should also be avoided wherever possible. It is always helpful to the reputation of the journal to include citations to previous papers published in ***Biomaterials***.

Figures and Tables

As noted earlier, experimental data should be represented in figures or tables wherever possible. Advice is not given here about the preparation of figures, detail being given in the guide to authors. Authors should note, however, that since figures and tables take up a considerable amount of space, they should be limited in number. Many authors used flow charts to represent experimental strategy or line drawings or photographs of equipment, most of which are unnecessary. Sometimes multiple figures are used with very little data on each, and which could be consolidated.

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Supplementary Data

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If the author is advised that the paper is rejected and cannot be published, the decision is final and not available for negotiation. This does not mean that the author is prevented from submitting further papers to the journal on a similar subject, but the authors are strongly advised to take into account any critical comments of the referees and any further papers will be considered as new submissions and submitted to the review process from the beginning.

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In this respect, it is essential that the authors are vigilant with the version of the paper that they are working with. It is not unusual to have authors submit a 'revised' paper, but only send the original version in error. This provides serious problems for the office.

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3. Brown W, Chow LC. Combinations of sparingly soluble calcium phosphates in slurries and paste as mineralizers and cements. US Patent No. 4612053, 1986.
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