CURRENT CONCEPTS REVIEW

BONE SUBSTITUTES IN 2003 : AN OVERVIEW

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The authors review the various bone substitutes which are currently available on the market place in Belgium. After describing the requirements for clinical use of such materials, they compare the biological and mechanical values of bone autografts, bone allografts, demineralised bone, xenografts, coral and synthetic materials such as calcium sulfate, calcium phosphate, ionic cement and bioactive glass. They stress the current paucity of data pertaining to the biological value of these materials and call for *in vivo* validation tests.

They aiso review biomolecules such as BMP-2 or OP-1, whose osteoinductive properties are currently under investigation.

Finally, they present the emergent field of cell therapy, in which osteoprogenitor cells are isolated from the patient's bone marrow and reinjected after *in vitro* cultivation.

They stress the therapeutic and medicolegal problems raised by the combination of medical devices, grafts, medicinal products and cells, all of which have a different status within the complex European legal framework.

INTRODUCTION

The number of bone substitutes available in Europe has increased sharply over the past few years. This development was driven by the new trends in minimally invasive surgery, particularly in the spine area where the need for bone grafts or substitutes is expanding.

However, the long-term efficacy of bone substitutes as an osteoconductive material remains controversial, and controlled clinical studies are still limited in number (16, 21, 24).

The ultimate goal of using bone grafts or bone substitute is to initiate a healing response that will produce new bone as an end product in an area where new bone is required. The site to be implanted must already contain enough osteogenic cells or must be enriched by a source of bone cells such as a bone autograft or autogenous bone marrow (5, 28).

To be considered as *osteogenic*, a material must contain osteogenic cells from the host. This requirement is only met by autogenous bone and by any material enriched with cultured autogenous bone cells.

To be considered as *osteoconductive*, a material must serve as a supporting structure for the host cells where they can migrate throughout, attach and differentiate into osteogenic cells. New bone formation within the scaffolding is the end result rather than a fibrous tissue scar. Unless it contains an osteoinductive factor, no material can be considered as *osteoinductive*.

There are two other very important requisites for successful bone formation to occur : *vascularity* and *mechanical stability*. These factors are largely surgeon-dependent and emphasise the importance of the surgical approach and the preparation of the site to be grafted (7, 9).

Any material considered for use as a bone substitute must meet the following requirements (16, 21, 24) :

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Table I. — List of human tissues, bovine tissues and bone substitutes available in Belgium.

Autograft				
Composition	Tissue bank	Forms	Remarks	Price /dose(Euros)*
Allograft				
Femoral head	human bone	tissue bank	variable	298- (full) [+ 70cc]
Femoral head	human bone	tissue bank	variable	174- (1/2) [+ 35cc]
Femoral head	human bone	tissue bank	variable	124- (1/3)
cancellous morcels	human bone	tissue bank	variable	100- (5cc)
cancellous morcels	human bone	tissue bank	variable	199- (15cc)
cortical bone powder	human bone	tissue bank	powder	75- (1cc)
cortical bone powder	human bone	tissue bank	powder	149- (3cc)
Medical device**	Composition	Company	Forms	Price (Euros)
Xenograft				
Isobone	bovine bone	DePuy	variable	134- (block 15×15×20mm)
Lubboc	bovine bone	Matthys	variable	118- (block 25×15×8mm)
Ceramic				
Tri-Calcium phosphate				
ChronOs	calcium phosphate	Matthys	variable	127- (granules-5g)
Bicalphos	calcium phosphate	Sofamor	variable	175- (granules-10g)
Vitoss	calcium phosphate	Cormed	variable	195- (granules-5g)
Biosorb	calcium phosphate	SDI	variable	148- (granules-5cc)
Hydroxyapatite				
Endobon	bovine bone	Biomed Merck	variable	92- (granules-5cc)
Biocoral	coral	Inoteb	variable	not available
ProOsteon 500	coral	Cormed	variable	147- (granules-5cc)
Technimed	synthetic	Comesa	variable	not available
Mixed(HA-TCP)				
Biocer	synthetic	DePuy	variable	not available
Triosite	synthetic	Zimmer	variable	208- (granules-5g)
OsSatura PCH	synthetic	Promed-Orthogèse	variable	112- (granules-2,5g)
Bioglass	synthetic	not available		
Other biomaterials				
Calcium phosphate cement				
ChronOs inject	synthetic	Matthys	injectable	not available
Biobon	synthetic	Biomet Merck	injectable	253- (5g)
Norian SRS	synthetic	undefined	injectable	not available
BioSource	synthetic	Strycker	injectable	not available
Calcibon	synthetic	Biomet Merck	injectable	not available
Calcium sulfate				
Osteoset	synthetic	Ortho-Service	pellets	125- (10cc)
Osteoset T(obramycin)	synthetic	Ortho-Service	pellets	455- (10cc)
Collagen + Ha (amorphous)				
Healos	bovine + synthetic	Sulzer medica	strips & disks	323- (per 2 strips)
Medicinal product**				
Differentiation factors				
OP-1 (BMP-7)	recombinant polypeptide	Strycker	injectable	4400- (vial)

* : Prices quoted in Euros according to verbal information in May 2002 or from data given by Unamec
** : Class to which the product or tissue belongs according to Belgian regulation

– It must be fully biocompatible,
– It must be able to serve as an anchoring surface for host cells,
– It must have a porosity that allows osteoconduction,
– It must be progressively resorbed and replaced by new bone (creeping-substitution).

CLASSIFICATION

The substitutes for bone replacement can be classified according to their origin or their purpose. The first classification will be used hereafter. Table I lists the bone substitutes and bone grafts currently available in Belgium.

Human

Bone autograft

Cancellous bone autograft remains the gold standard to which every substitute must be compared. Cancellous bone has a major advantage in that it supplies not only a bone volume but also osteogenic cells that are capable of quickly laying down new bone. The current drawbacks include donor site morbidity (pain at the site of the procurement, potential for local complications such as haematoma, fracture etc…), and the limited availability of autogenous bone (7, 11).

Bone allograft

Allograft is the bone substitute most frequently used in Belgium, owing to the good organization of tissue banks in the country. Belgium was the first country in Europe to have a specific legal framework concerning tissue banking in 1988 (10). The most current allograft usually originates from femoral heads that are collected during primary hip arthroplasties and subsequently preserved.

The bone can be made available in freeze-dried or deep-frozen forms. The processing of bone is an option where all the cellular debris and bone marrow are removed from the native bone. This processing is favored, as it has been shown that delipidation promotes the osteoconductive capacity of the processed bone and reduces the immune response to the allograft (31). Evenmore, it has been shown that a bone allograft can be an appropriate vehicle for the local delivery of antibiotics (36). Unpublished data from our laboratory have shown that rifampicin-impregnated bone after a 6 month-freezing period can release the antibiotic at an active concentration for at least three weeks following implantation (unpublished data).

Mechanically, the strength of bone will be greatly influenced by the mode of preservation and sterilisation. Freeze drying alone and deep freezing do not alter the mechanical properties of a bone. Irradiation of a freeze-dried bone allograft will cause a significant loss of the original strength whereas irradiation of a deep-frozen allograft will not. Consequently, a freeze-dried and irradiated bone is not appropriate for structural reconstruction (8). This kind of processed bone must be used in an area that will be mechanically protected, with or without osteosynthesis. One of the most appropriate indications for freeze-dried and irradiated bone is its use as bone morsels for impaction in a contained cavity. Such morsels should be freed from cartilage if prepared for impaction (2).

Bone allografts can be sterilely procured or secondarily sterilised, usually by irradiation. At 25 KGy, bacterial sterilisation is achieved if the bone has been properly managed before the final sterilisation. However, this dose is not virucidal for the human immunodeficiency virus (HIV) (29), for which risk prevention should rely on tissue bank screening procedures and inactivating treatments.

The theoretical risk of virus transmission for HIV is less than one chance out of a million and for the hepatitis C virus (HCV), one chance out of 200 000 for a deep-frozen, non-irradiated and unprocessed bone that has been procured from a selected and serologically screened donor. This risk is further decreased after a 6-month quarantine, to less than one out of a billion for HIV and one out of 2 million for HCV. If a validated tissue processing is used, this concern is virtually eliminated.

Demineralised bone

This particular form of bone remains quite popular in the USA and is becoming more and more

popular in Europe because demineralised bone not only fills space but is also osteoinductive ; in other words, it will be able to recruit host cells to become osteoblasts (16, 25). The end result of such a graft should be the formation of a new bone ossicle made by the induced osteoblasts from the muscle (13, 33, 34). In theory, this type of graft is the most biologically active among the bone allografts, as it will transform uncommitted cells from the host into osteoblasts.

To augment the available volume of demineralised bone, manufacturers may add some chemical agents such as glycerol to increase the fluidity and the injectability of the bone, or calcium sulfate. However, such an addition of chemicals may have a potentially deleterious effect as it has been shown that glycerol might cause acute renal failure in small laboratory animals (2, 35).

A newer trend is to add some bone derivatives to demineralised bone, such as collagen fibers, in order to enhance the plasticity of the material. Some are moldable and harden when warmed to body temperature. Demineralized bone is currently available in the USA as gels, putties and pastes (16). These are not yet available in Belgium.

The efficacy or in other words the osteoinductivity of these bone grafting materials remains questionable. There are very few data provided by the producers or the tissue banks to demonstrate osteoinductivity (13). This proof of claim is costly and needs either an *in vivo* or *in vitro* assay that should be repeated for each batch produced. This should be required and viewed as a quality control procedure.

Animal

Xenograft

Today, bone xenograft has nearly been abandoned in Belgium because of the emergent incidence of health risks carried by animals. There are two xenografts available in Belgium (Isobone®, Lubboc®). These grafting products have been thoroughly treated with solvents and other proprietary processings in order to make them safe. However, there are no data in the literature to support their incorporation and hence their transformation into living bone. Most often, a xenograft elicits a sustained immune response that results in its sequestration by a thick layer of fibrous tissue (32).

Hydroxyapatite can be obtained by thermal treatment (1200-1300°C) of bovine bone (Endobon®). During this procedure, the organic matrix is lost and leaves only the mineral phase. The original porosity of the bone is maintained. Another kind of xenograft is made from bovine dermal collagen that is associated with noncrystalline hydroxyapatite for grafting purpose (Healos®).

Coral

This material is derived from natural corals and as such is also a xenograft. A coral is also a kind of ceramic as it consists of tricalcium carbonate. The main interest is that it offers a skeleton that is able to be invaded by human cells ; it has been used in bone surgery for one decade (22) and it can also serve as a carrier for other substances (32). Coral is available in various preformed shapes. There are two chemical forms available :

– Calcium carbonate (Biocoral®) : it has a pore size about 200 µ and a porosity volume of 20% which is considered optimal for bony incorporation. The natural interconnecting porosity is respected. It is resorbed and replaced by new bone.
– Hydroxyapatite (ProOsteon 200 & 500®) : this is obtained by a thermal and chemical treatment of the original calcium salt. The advantage of this kind of hydroxyapatite is that it retains the porous microstructure of the coral without organic compounds. ProOsteon® is marketed in several forms with two pore sizes.

Synthetic

Calcium sulfate

This compound is well known as plaster of Paris and serves as a cast constituent for fracture immobilisation. In the presence of water, it forms a moldable paste that will harden gradually. Its use as a carrier and a bone filler has also been revisited over the past decade. Calcium sulfate may be

used as a slow-releasing antibiotic reservoir (4, 27) (Osteoset T®) or as a bone defect filler (23, 26, 30) (Osteoset®). *In vivo*, calcium sulfate is resorbed in about 6 to 8 weeks without an inflammatory response. The plaster has no significant mechanical capacity and cannot be used as a structural material for bone. Calcium sulfate has no osteoconductive capacity as it is rather quickly resorbed.

Calcium phosphate

This mineral salt is the largest member of the ceramic family (21, 24, 30). It is close to the bone mineral as it has a very similar composition. Ceramics are obtained by sintering at very high temperature (> 1000°C). The initial powder is compacted for shaping while porosity is given by the addition of a thermolabile agent. The average porosity of the ceramics is about 35 % with interconnected pores. Among the advantages of these substitutes, there is the chemically favorable environment for bone cells that deposit new bone directly onto the ceramic. They are fully biocompatible. Among their drawbacks, they are rather weak in terms of mechanical resistance.

There are two basic calcium phosphate ceramics : hydroxyapatite and tricalcium phosphate. Hydroxyapatite is hardly (almost not) soluble and its resorption takes years and it will consequently remain radiographically visible for a long time. Whether this is clinically important is at present unknown.

Hydroxyapatite can be obtained from three sources : by chemical synthesis (Technimed®), by sintering of animal bone (Endobon®) or from coral (ProOsteon®).

Tricalcium phosphate is more soluble and its resorption occurs in months (ChronOs®, Bicalphos®, Vitoss®).

The combination of both products is available and has the advantage of the different resorption rates (Biocer®, Ossatura PCH®, Triosite®).

Ionic cement

Calcium phosphate cement has been in clinical use for several years (Biobon®, BoneSource®, Calcibon®, Norian SRS®, ChronOs inject®). It is an association of various calcium salts which are dissolved and mixed before use to form an injectable paste. The paste hardens within minutes at ambient temperature and pressure (6, 21, 24, 30). It is slowly resorbed and replaced by new bone but it is not truly osteoconductive as it only has a very small pore size (4 μ). New bone can be deposited on its outer surface. Calcium-phosphate cement can provide mechanical support in comminuted fractures or an increased strength to hold fixation devices in osteoporotic bone. The material is rather strong in compression but is weak in torsion and shear.

Distal radius fractures have been investigated as a target indication for Norian cement approval. The cement has been found to be as effective as wire fixation in a postmortem study (37). In clinical studies of distal radius fractures, Norian cement was found as effective as other treatments but it could not prevent loss of reduction when used alone (18, 19, 20).

Bioactive glass

This chemical family is characterized by a high content in silica, calcium and disodium oxide. Calcium and silicate ions are progressively released and interact with the surrounding cells (12). Once implanted, the outer surface will form an amorphous layer of calcium and phosphate that binds to proteins, collagen, fibrin and growth factors. The material can be made porous or plain. It is gradually resorbed . Like calcium phosphate, bioglass cannot offer structural support in bone surgery. This kind of bone substitute is not yet available on the Belgian market.

ASSESSMENT AND PROOF OF BIOLOGICAL CLAIMS

For most products, a set of experimental data is available that allows to delineate their main characteristics. However, it remains difficult for the surgeon to determine which product best fits a specific indication. Most often, there is no available comparison between substitutes or between a substitute and an autograft.

There is clearly a need to have an *in vivo* validated assay to assess and compare the osteoconductive property of a bone substitute with that of a graft in a way that mimicks the surgical environment. The only test that has been set up is in the rat, but the size of the test permitted remains far from the usual sizes in an operating room (1). One of the objectives of the European Association of Musculoskeletal Transplantation (EAMST) is the validation of an *in vivo* test with which every bone substitute could be tested and compared in a reproducible manner.

Osteoinduction can be verified by the new bone formation after implantation in the dermis or the muscle in rats (33, 34).

DIFFERENTIATION AND OTHER BIOLOGICAL FACTORS

These biomolecules are not bone substitutes by themselves. However, they are able to participate in the process of new bone formation by triggering one or several steps of a cascade that leads to ossification (15, 33).

To be effective, these molecules need to be carried to the site of new bone formation and to be released appropriately. Furthermore, most often they need the presence of other cofactors in order to fully express their action. Recombinant bone morphogenetic protein-2 (BMP) from Wyeth-Ayerst Laboratories and OP-1, a recombinant BMP-7 from Stryker Biotech are the most promising molecules, but their current indications and doses in various musculoskeletal disorders await further investigations as comparative studies with demineralized bone could not demonstrate a superiority of the recombinant human osteogenic protein (10).

CELL THERAPY

This is another emergent area dealing with cells from the patient. This approach includes the procurement of cells from a patient, the selection of the desired phenotype and its further expansion in laboratory. Thereafter, the cultured cells are transferred back to the same patient. This therapy is starting to be promoted by some companies or tissue banks. In orthopaedic surgery, chondrocytes and osteoblasts are the targeted cells. Osteogenic precursors can be isolated and further cultivated from the iliac bone marrow (5, 14, 17, 28). This treatment will in the near future be an option for nonunions and cartilage defects.

COMBINED BONE GRAFTING MATERIAL

There will be more and more associations between medical devices, grafts, medicinal products and cells. These combinations will offer a vast array of treatment options for the surgeons. These interactions will add a level of complexity not only from a legal point of view (which regulations are being applied ?) but also from a therapeutic one (which biological principle is most effective ?).

INDICATIONS FOR BONE SUBSTITUTES IN 2003

The main indication for bone substitutes is the filling of an osseous cavity or the augmentation of bone to enhance its mechanical strength. Nonunions and arthrodeses in an extraskeletal bed are exclusively restricted to osteogenic or osteoinductive material.

LEGAL FRAMEWORK IN BELGIUM

Today, the legal framework for bone grafting materials and biomaterials is puzzling in Europe and to some extent in Belgium. Recombinant osteoinductive proteins are considered as a medical device in the USA and as a medicinal product in Europe. Similarly, a bone graft is considered as human tissue in Belgium and France and as a medicinal product in Germany.

The situation in Belgium can be summarized as follows : there are three basic classes to be considered for regulation : a medical device, a human tissue and a medicinal product. A ceramic is a medical device and as such needs a EC (European Com-

muniyy) mark whereby the manufacturers proves the efficacy and safety of the product. After being EC marked, the ceramic can be distributed in Belgium. Similarly, a processed bone from animal origin is a medical device that only needs a EC mark. Human tissue is considered as such in Belgium and is regulated through the health ministry. It must be distributed in our country by a registered tissue bank. The registered bone banks in Belgium can be found on the website of the Belgian public health ministry : www.health.fgov.be/AGP/fr/sang/banques-tissus.htm.

If not available in Belgium, a tissue can be imported only through a tissue bank after being registered at the health ministry. A medicinal product is regulated at the European and national level.

CONCLUSIONS

A very large panel of bone substitutes is offered today to the orthopaedic surgeon. In Europe and in the USA as well, bone itself remains the preferred substitute for bone. This will be challenged in the near future by other, more complex substitutes such as demineralized bone, interactive association of a medical device and grafts or cells. It might be speculated that there will be a slow shift from a tissue approach to a more cellular approach.

These types of substitutes are useful for small contained bone defects but not for large bone defects where structural allografts or autografts are mandatory. Unless truly osteoinductive, all these substitutes need a well-vascularized bone bed whose preparation is surgeon dependent.

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