The Combination of Apatite-Wollastonite Glass Ceramic and Bone Morphogenic Protein

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ABSTRACT

The clinical application of synthetically granu lated hydroxyapatite (HA) in the patients with bony defects as a bone substitute has universally accepted that HA can directly bond with the host bone. Another type of bioceramics, Apatite-Wollastonite (A-W GC), which already has been proved to be as effective as HA when embedded in body fluid, has higher mechanical property. Hence, A-WGC should have wider clinical application.

Bone Morphogenetic Protein (BMP), isolated from bovine and human catical bone has been proved to be capable of inducing mesenchymal cells to form cartilage and bone tissue in human quite effectively. However, the delivery system of BMP is still unknown.

This paper was intended to propose the two possible methods of combining the two good biomaterials, HA and BMP, so that they can be used to replace the bone loss and at the same time inducing new bone formation in a short period.

1. Introduction

Reconstruction of a local bone defect resulting from trauma or surgical resection of a bone tumor is a major problem in orthopaedic and maxillofacial surgery. When the defect is small, allogenetic bone grafting is the best choice, although its use involves a second operation. However when the defect is large, as in wide resection of an osteosarcoma, allogenetic bone grafting or use of a synthetic prosthesis with tissue-compatible alloys or ceramics is sometimes considered. There are, however, problems with both alternatives. Antigen removal by physical or chemical treatments and a bone bank system are thought to be solutions to problems of allogenetic bone grafting, but the bone bank system has problems of limited resources and large expenses. Synthetic bone substitutes have been tried in a few cases.

In cases with large bone defects, unlike ordinary hip and knee arthroplasties, special biomechanical conditions must be considered. For example, when part of the midshaft of a long bone is replaced by a block of solid biomaterial, the prosthesis must function not only as a spacer but also as a fixation device with a small contact area to the bone, which is subjected to high shearing and compression stresses at the interfaces between the bone and prosthesis when loaded. This situation may induce growth of fibrous tissue at the interfaces, resulting in loosening of the prosthesis, this undesirable situation (refered to as dynamic loading of the prosthesis by Ducheyne et al.) is improved when the prosthesis is encased within a newly formed bone mass bridging the ends of the two bones (static loading of the prosthesis). In this way the prosthesis reaches a stable condition without loosening. This technique was successful in clinical cases with small defects. However, a similar improvement would be unlikely in cases with large defects in the long bones because of the limited amount of bone mass formed by osteoconduction. Thus, a new method is needed to enhance new bone formation to resolve this problem.

In terms of the location of bone formation, the surface of the spacer should be regarded as a heterotopic site If ectopic bone could be produced by osteoinduction on the surface of the spacer in a shorter period, and could connect the ectopic bone to the bone at both ends, the formation of bone bridges

and repair of bone defects would be more rapid. This idea prompted the author to start this study using bone morphogenetic protein (BMP) in combination with solid biomaterials.

In the past decade, many synthetic biomaterials typed bioinert ceramics such as alumina ceramics, and bioactive ceramics such as calcium phosphate ceramics, Bioglass (Na_2O-CaO-SiO_5-P_2O_5 glass), Ceravital (apatite-containing glass ceramics), and A-W GC (Apatite-Wollastonite containing glass ceramics) have been developed for bone substitues. Since alumina ceramics do not form a direct bond with bone, there is a high possibility for alumina implants to become loose after a long period. Bioactive ceramics are reported to form a tight chemical bond to bone, but except for A-W GC they have a relatively low mechanical strength. Their utility is thus limited to non-load-bearing structures or to filling bone cavities. In contrast, A-W GC has a greater mechanical strength than that of human cortical bone or other bioactive ceramics. Prof.Yamamuro et al reported that the strength of its bonding to bone is significantly higher than that of other bioactive glass ceramics, and that it is possible to use A-W GC for implants that will be subjected to load-bearing. Clinically, they have already used A-W GC as an artificial vertebral body, which has been successful for many years.).

However, it is difficult to mold ceramics to the size and shape of various bone defects because of their brittleness. If the implanted ceramics do not fit the bone defects, they may fail to bond to bone in spite of their osteoconductive potential. The use of a biological material, which can be shaped and molded in situ to conform to both the bone and the implant, and which have capability to induce bone formation, would secure firm and fast fixation of the implants even if without an initial precised fit. Bone morphogenetic protein (BMP) has an excellent osteoinductive potential. It can be used to fill the defects between the bone and implant.

It is well established that a bone morphogenetic protein (BMP) isolated from bovine and human cortical bone matrix induce mesenchymal cells to form cartilage and bone tissue. Currently, basic studies on BMP are focused on the chemical nature of BMP as well as its induction mechanisms.

The potential for BMP is its capacity to initiate the process of bone induction from the onset of development. This differs from the process of transplantation of massive bone grafts that do not maintain viability and may require years for incorporation and substitution. Hypothetically, BMP may induce bone marrow stroma cells and perivascular connective tissue cells from the host to grow across the defect and thereby supplement the ingrowth of previously differentiated osteo-progenitor cells.

However, from a clinical point of view, utilization of BMP to form new bone and for regeneration of defects is urgently needed. An extremely small quantity of BMP occurs in whole bone matrix. Therefore, for the purpose of clinical bone reconstruction surgery implantation of demineralized bone matrix itself is not sufficient. An effective BMP delivery system from a synthetic vehicle for concentrating BMP is required. In addition, a graft material that shapes the bone or supports massive defects is desirable. If the material is gradually resorbed and replaced by induced bony structure, clinical bone reconstruction may proceed most promisingly.

2. Literature Survey of BMP and A-W GC

BMP is a noncollagenous protein (NCP) extracted biochemically from cortical bone matrix, dentin, and murine osteosarcomas. BMP is a cell differentiation inducer, assayed both in vitro and in vivo, and has the capacity to switch the pathway of development of perivascular connective tissue cells into cartilage or bone, or both. Basic science investigations on bone matrix and BMP-induced bone formation by Urist, Urist and Iwata, and Urist et al.may lead to practical applications in orthopedic and dental surgery.

Bone morphogenetic protein (BMP) is reportedly responsible for bone induction,i.e.,postfetal cytodifferentiation of mesenchymal cells into bone tissue through endochondral bone formation. Due to the rather hydrophobic and water-insoluble nature of this protein, bone formation in classical experiments of bone induction occurs as the original implants of decalcified bone matrix or devitalized osteosarcoma tissue are biodegraded and replaced in situ by new bone induced in the ectopic sites.

BMP is water soluble following some purification steps that probably remove proteins that couple to BMP, causing it to be insoluble following dialysis against water. In invitro systems, the water-soluble BMP fraction has been reported to stimulate DNA synthesis and cell replication in organ cultures of fetal rat calvariae. However, little is known about the effects of purified, water-soluble BMP on osteogenic cells in orthotopic bone in vivo. This might be attributable to the fact that implantation of water-soluble BMP fraction alone in vivo probably results in rapid diffusion of BMP away from the implant site so that its local concentration is insufficient to stimulate cartilage and bone formation. BMP's water solubility prevents its effective and continuous inductive action on orthotopic bone in vivo. The use of a carrier of water-solution BMP that prolongs its release and thus maintains its local concentration would be desirable.

In nature, BMP is found in an aggregate of several insoluble proteins, e.g., osteonectin, matrix

glutamic acid (GLA) protein, histones, and calmodulin. BMP has been isolated in a water-soluble form but has not yet been purified of all trace proteins.

Partially purified,i.e., 95% bioactive, BMP has the characteristic of an acidic protein. BMP is insoluble in acetone, absolute alcohol, chloroform, methanol, and triton X-100. It is disulfide-bonded and is inactivated by heat greater than 70. It is deaminated in HNO-3, B- mercaptoethanol reduction, lathyrogens, penicillamine, and ultrasound. BMP binds to hydroxyapatite in a solution of 6 mol/liter urea at a pH of 7.2. BMP is resistant to collagenase, chondroitinases A,B,C, amylase, neuraminidase, hyaluronidase, alkaline phosphate, acid phosphatase, chymopapain, tyrosinase, and thermolysis, It retains its activity after limited proteolysis for one hour with pepsin or trypsin. The quantity of BMP in human cancellous bone is too small for detection but the hBMP in marrow-free cortical bone represents approximately 0.001% of the wet weight.

Water-soluble hBMP is an acidic protein. Usually the donors were victims of accidental injury and designated as normal, healthy individuals. BMP has also been isolated from osteosarcoma, Ewing's sarcoma, chondrosarcoma, and other cellular tumors. High serum BMP radioimmunoassay levels have been detected in growing children and in patients with Paget's disease. Low levels have been reported in patients with severe osteoporosis. hBMP in association with other insoluble noncollagenous proteins (hBMP/iNCP) was prepared and partially purified by the following four-step procedure : (1) gelatinization and extraction of relatively soluble noncollagenous proteins; (2) differential precipitation of insoluble noncollagenous proteins in a solution of 0.5 mol/liter guanidinium HCl ; (3) extraction of gelatin peptides in a buffered citrate solution; and (4) removal of osteonectin-type proteins in Triton x-100. The product is an aggregate of low molecular weight non-collagenous proteins relatively insoluble in water at 2. The insolubility of the BMP is attributable to the complex formation of a matrix-carboxyglutamic acid-containing protein (MGP), possibly through a Ca^{+2} bridge. When dissolved from Ca^{+2} and the MGP carrier by 50 mol/liter edetic acid, the carrier-free BMP becomes water-soluble.

Hanamura et al. demonstrated that BMP could be extracted under dissociative conditions with 4 M guanidine hydrocholoride from mouse osteosarcoma tissue or rat bone matrix. Urist et al. devised a 2-M $CaCl_2$-6-M urea solvent mixture for extraction of BMP from bovine bone in kilogram batches. Since the development of these new methods of BMP extraction, research on the osteoinductive protein has rapidly advanced.

Metals and plastics have been used primarily as implant materials for bone and joint replacement for a long time. Recently, much attention has been paid on ceramics because of their good compatibility with living tissues. Among them, bioinert-alumina ceramics are already used practically. They show high mechanical strengths and good biocompatibility. These materials are used for femoral heads of total hip-replacement prostheses, for segmental replacements of long bone, and for vertebral body replacement (Kyocera, Kyoto, Japan). However, alumina-ceramics do not form a chemical bond with osseous tissue. Therefore. the prostheses must be fixed to the bone by mechanical means, and the problem of loosening over a long period becomes critical.

Bioactive ceramics in general have a character of directly bonding to the bone in vivo, but they are mechanically weaker than bioinert ceramics. Therefore, for their clinical application, bioactive ceramics require different methods of utilization from those of the bioinert ceramics. Typical bioactive ceramics include Bioglass, Ceravital, tricalcium phosphate, hydroxyapatite and A-W glass-ceramic. They differ from each other considerably in their mechanical strength, chemical properties and biological reactions.

Bioglass (Na_2O-CaO SiO_2-P_20_5 glass) and Ceravital (apatite-containing glass ceramics) have been reported to form tight chemical bonds with osseous tissue. Their mechanical strength, however, is not sufficient for use in bone prostheses, so their clinical application has been limited mainly to problems of the middle ear. The stems of total hip prostheses, other metal implants, alumina implants, and dental implants, coated with Bioglass or Ceravital, have been used under load-baring conditions in experiments on animals. Calcium phosphate ceramics (hydroxyapatite and tricalcium phosphate) have received wide attention. Their chemical properties resemble those of bone mineral. However they have low mechanical strength. Their utility is limited to non-weight-bearing structures or to filling cavities in bone.

A glass-ceramic containing apatite and wollastonite (A-W GC) been reported to have a relatively high mechanical strength as well as the capability of forming a strong chemical bond with osseous tissue under non-weight-bearing conditions. Although the mechanical strength of this glass-ceramic is lower than that a alumina, it is higher than that of Bioglass, ceravital, or hydroxyapatite. This glass-ceramic may be useful as a material for load-bearing prostheses.

The major problem with ceramic is its brittleness. Glass and ceramic materials fail mainly because of the stress-dependent growth of preexisting flaws to dimensions which are critical for spontaneous crack propagation. Ceramics have two fatigue

phenomena. One is static fatigue under a static stress. The delay time required for fracture under a static load is the time required for a microscopic flaw to grow to the critical size necessary for spontaneous crack propagation. The time/failure ratio decreases with increasing stress. The other is dynamic fatigue : fatigue under dynamically stressed conditions. Fracture strength decreases when the stressing rate becomes low in a moist environment. Since fracture flaw has more time to grow from a subcritical to critical size at a low stressing rate, the speed of the reaction between the ceramics and water at the tip of a crack is faster than that of a fracture flaw, Thus, the ceramics will fracture in low stressing rate. The data under dynamic loading give an alternative method of characterizing the stress-corrosion susceptibility of ceramics.

From the results of the aging test and the dynamic fatigue test, it is concluded that the reduction of mechanical strength in A-W GC and HA will not occur in living tissue free of stress.

Glass-ceramic implants containing apatite and wollastonite were studied under load-bearing conditions in a segmental replacement model in the tibia of the rabbit. The result showed that the load to failure of glass-ceramic implants containing apatite and wollastonite increased with time. On the basis of mechanical strength and the performance of the bone-implant interface, prostheses fabricated from glass-ceramic containing apatite and wollastonite should be usable under load-bearing conditions.

Recently there has been much research concerning surface reactions of surface active ceramics. A Ca-P-rich layer has been discovered on surface-active ceramics. The apatite layer may result from Ca and P ions in living tissue. A chemical bond could occur between the mineralized matrix of bone and the apatite layer of bioactive ceramics. Walker indicated that surface area played an important role in bonding to bone.

Jarcho reported that the surrounding physiological media would ultimately produce calcium phosphate solids on all calcium phosphate materials in the form of biological apatite. The storage of P may be partly formed by the surrounding media. However, the Ca-P-rich layer may result from the dissolution of glass-ceramics according to results of SEM-EPMA observation. Hench reported that the Ca-P-rich layer stabilized at about 30 μm after 3 months, while the Si-rich layer continued to thicken to 240 μm over 28 months from Bioglass to bone as observed by SEM-EPMA. Holand indicated that the Ca-phosphate-rich interface layer, which has apatite crystals and a thickness of about 5-10 μm 16 weeks after implantation, grew between machinable bioactive glass-ceramic and bone through solid-state reaction. Ito and Kokubo tested apatite crystal formation and changes in the x-ray intensities of Ca,P,Si and Mg on the surface of A-W GC after soaking them in physiological saline. Fluorescent x-ray analysis revealed a remarkable change in the x-ray intensity of P on the surface of A-W GC; that of Mg and Si decreased, and that of Ca had little change. Apatite crystal formation was observed on the surface of A-W GC by x-ray diffraction. Studies by Yamamuro et al revealed that the reactive zone of Apatite and wollastonite glass-ceramics stabilized 6 months after implantation. This observation may show that the dissolution of glass-ceramics did not occur. These results are beneficial for clinical application since glass-ceramics must not break in a body fluid environment. However, it is not know whether the apatite layer is crystal or not. The apatite layer may be amorphous in its early stage. It is very difficult to evaluate the apatite crystal using x-ray diffraction in vivo since the apatite layer between bone and ceramics is very small.

3. Delivery systems of B.M.P.

1. Natural delivery route of BMP

a) Matrix v-carboxyglutamic acid protein (MGP).

Sato and Urist reported that an aggregate of MGP with BMP retarded resorption and displacement of BMP from the host bed.

b) Collagen,

with respect to collagen, the same problems as MGP remain to be solved.

c) Calcium phosphate.

Physiological bone apatites, CO_3 apatites, are soluble and can be metabolized easily, sintered apatites remain in the body without being metabolized.

2. Other experimental delivery routes of BMP

a) Sintered calcium phosphate.

Tricalcium phosphate (TCP) and hydroxyapatite (HA) are widely used as bone substitute in the field of orthopedics and dentistry, generally the form of sintered preparations. TCP and HA are nonbiologic, nonimmunogenic materials but have no osteoinductive or bone-reparing ability, except that of guided bone formation from the host bone. As a BMP delivery system, TCP may be better than HA because HA is not soluble in extracellular fluid in vivo. Urist et al. suggested that further research is necessary to find a sintered calcium phosphate formula that can be resorbed and replaced by bone more rapidly and easily than TCP. If carrier materials are not completely resorbed, they may disturb bone remodeling in the implanted area and caused difficulty in the functioning of normal bone.

b) Fibrin.

Human plasma, the two major components of which are fibrinogen and thrombin, prepared in the form of fibrin sealant (FS) is used as an adhesive in

caridovascular surgery, nerve surgery, orthopedic surgery, and abdominal surgery. Use of its biologic and physiologic properties, fs seals wounds, adheres to living tissues, and provides hemostasis. Extensive research on FS demonstrates many possible applications to orthopaedic surgery. Fibrin is an organic, biodegradable material derived from human plasma. Clinically, fibrin is relatively nonantigenic. Furthermore, fibrin has angiotropic, hemostatic, and osteoconductive properties. From all points of veiw, human fibrin could prove to be a good biodegradable distributor-carrier for BMP. If the composite of BMP and fibrin has osteoinductive capability in patients, it might be utilized in treatment of fractures and as an osteoinductive glue in the form of FS.

c) Collagen or Gelatin

From experimental animal studies. Implantation of water-soluble BMP fraction alone resulted in no formation of either ectopic bone or periosteal bone in adjacent bone. On implantation of water-soluble BMP fraction with collagen (BMP/collagen), both ectopic and periosteal bone formation were consistently observed, whereas in implantation of BMP with gelatine (BMP/gelation), only periosteal bone formation was observed. These differences may be explained as follows : implantation of a small amount of water-soluble BMP alone might result in rapid diffusion of BMP so that its local concentration did not remain sufficient for induction events to occur. Gelatin, which is water soluble, was rapidly absorbed and released the BMP, which caused periosteal bone formation. However the concentration soft BMP did not remain high enough and/or long enough to induce mesenchymal cells to form ectopic cartilage and bone. In contrast, collagen, which is water insoluble, retained some BMP molecules, delayed its delivery to responding cells, and also acted as a scaffold for ectopic bone formation. At the same time, the collagen released some BMP that diffused to the periosteum of adjacent bone to cause periosteal cartilage and subsequent bone formation.

d) Non-immunogenic Polymer Carrier (Polyacetic polyglycolic acid-polymer) BMP, in an aggregate of bone matrix NCP, mixed with-granular PLA/PGA. Investigations are in progress on new delivery systems that are resorbed more rapidly than PLA/PGA and release the BMP as the host grows into the bone defect. In this respect, bone matrix/iNCP is a natural BMP carrier. In experimental animals PLA/PGA-delivered BMP induces bone formation across the periosteal surface outside the defect. Depending on the quantity, the delivery system is reabsorbed gradually over periods of weeks or months. Inside the defect unresorbed remnants could become a barrier, and therefore the PLA/PGA must be applied as an onlay implant outside while the marrow stroma fill the inside of the defect.

e) Plaster of Paris (PLP)

Plaster of Paris (PLA) has long been used as a filling material of bone defects and is reported to be resorbed rapidly in vivo. The PLP did not cause any foreign body reaction and may be a potential bone substitute. In the experiment a PLP preparation with BMP was almost completely resorbed and induced lamellar bone with bone marrow within six weeks. PLP was different from MGP, collagen, TCP, or HA because it did not influence resorption and displacement of BMP from the host bed. When compared with the resorption of the PLP composite, a larged amount of PLP of the control remained at later periods. Although the reason for this is unknown, it may be related to the difference between PLP densities of the control and the BMP/PLP composite. However, further studies are necessary to learn the practical applications of a BMP/PLP composite. There are several problems. The first one is heat, which is generated when PLPA is cured and may influence the physiologic activity of BMP. The second is the response to a large bone substitue mass of BMP/PLP composite in humans. Finally, the influence on calcium levels in blood serum by resorption of a PLP implant must be considered. When such problems are overcome, PLP may be hailed as one of the ideal delivery systems in clinical application.

4. Possibilities of combination between A-W GC and BMP.

Many studies have shown the good prospects and characters of Apatite-Wallasonite bioactive ceramic and the importances of Bone morphogenic Protein in inducing new bone formation, as already mentioned. If the combination of these two promising materials could be prepared and could function effectively, an enormous benefit would be expected when they are to be applied in indicated clinical situations.

These are the proposed methods for the combinations of BMP and A-W GC

1. By virtues of surface HA layer, created by A-W GC immersed in actual or simulated body fluid.

1.1 Chemical bonding. This may occur because the HA. is known as a carrier which combines proteins, nucleic acid, enzymes and has specific affinity to BMP.

1.2 Chemical and mechanical binding. BMP is known to be dissolved in phosphate buffer solution with urea. If the A-W GC with creative layer of HA is immersed in the solution, the HA layer would absorb the BMP deeply. Then the buffer was removed by dialysed against water and would produce precipitate on and inside HA layer

1.3 Mechanical binding. BMP is suspened in cold distilled water and A-W GC directly immersed in it, then lyophilized.

2. A-W GC - BMP composite with fibrin as a carrier.

3. A-W GC - BMP composite with collagen as a carrier.

4. A-W GC - BMP composite with polyacetic polyglycolic acid as a carrier.

5. A-W GC with BMP - plaster of paris composite

6. A-W GC porous surface, directly combines with BMP.

Extensive studies should be performed in order to investigate the possibilities of these different methods of combination between the BMP and A-W GC Should they likely to be successfully combined, the pros and cons of each individual have yet let to be determined.

5. Conclusion

A-W glass-ceramic is the representing bioactive ceramic which can be used for substituting pathological lesions in the human skeleton. Hydroxyapatite granules have successfully been used, for many years in Japan to fill in the bone defect remaining after excision of large bone tumors. However, it usually takes several months for the hydroxyapatite to be firmly and directly bound to the newly formed surrounding bone. On the other hand, in vitro and in vivo studies revealed that A-W glass- ceramic crystallizes a bioactive apatite layer on its surface much earlier than the hydroxyapatite in the tissue fluid. In addition, A-W glass-ceramic has greater mechanical strength than hydroxyapatite, and hence, the former seems to have wider clinical applicability than the latter.

Although it is generally assumed that BMP could be valuable adjunct to bone and joint surgery and dental oral surgery, the ideal delivery system has not yet been found. The yield of highly purified BMP is extremely small in relation to the weight of bone used as starting material. If a surgeon wishes to repair a large bone defect or fracture site, a great amount of BMP would be needed. If the defect were implanted with purified BMP, a quantity sufficient to diffuse throughout the target perivascular mesenchymal cells or stromal cells of bone marrows would be required. Consequently, the problem is how to distribute the BMP to obtain optimum osteoinductivity with the smallest quantity of implant.

In the coming future ultimate proof efficacy of the combination BMP inducing bone formation and A-W glass ceramic - as a scaffold or defect filler to repair bond union and segmental defects must ultimately be demonstrated in a prospective, randomized, double-blind investigation of a consective series of cases. Eventually these investigations will be extended in a multi-institutional analysis. If the implantation proves to be a significant advance, the success in orthopaedic surgery for non-unions and large segmental defects could be significantly improved.

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