Design of bone-integrating organic-inorganic composite suitable for bone repair

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1. ABSTRACT

Several ceramics exhibit specific biological affinity, i.e. direct bone integration, when implanted in bony defects. They are called bioactive ceramics and utilized as important bone substitutes. However, there is limitation on clinical application, because of their inappropriate mechanical properties such as high Young's modulus and low fracture toughness. Novel bioactive materials exhibiting high machinability and flexibility have been desired in medical fields. Mixing bioactive ceramic powders and organic polymers have developed various organic-inorganic composites. Their mechanical property and bioactivity are mainly governed by the ceramics content. It is known that bioactive ceramics integrate with the bone through bone-like hydroxyapatite layer formed on their surfaces by chemical reaction with body fluid. This is triggered by a catalytic effect of various functional groups. On the basis of these facts, novel bioactive organic-inorganic nanocomposites have been developed. In these composites, inorganic components effective for triggering the hydroxyapatite nucleation are dispersed in polymer matrix at molecular level. Concept of the organic-inorganic composite is also applicable for providing polymethyl methacrylate (PMMA) bone cement with the bioactivity.

2. WHY ORGANIC-INORGANIC COMPOSITE IS NEEDED?

When a part of our bone is lost due to diseases and accidents, the damaged part must be filled with a substitute. For this purpose, bone transplantation is most widely used in medical fields. There are two types of the transplantation, autograft and allograft. In the former, bone grafts are extracted from healthy bone tissues of the patients themselves. In the latter, they are extracted from the other patients. However the bone transplantation still has problems of unstable supply, invasion into healthy tissues to extract the bone grafts, contamination with some disease factors.

Therefore artificial materials with ability to function as bone tissue are most desirable for bone repair. However, artificial materials implanted into the bone defect are generally encapsulated with a fibrous tissue of collagen. This is a normal reaction for protecting our living body from foreign substances. Because of this phenomenon, the implanted materials are isolated from the surrounding bone and do not integrate with the living bone. This causes severe loosening of the interface between the implants and the bone
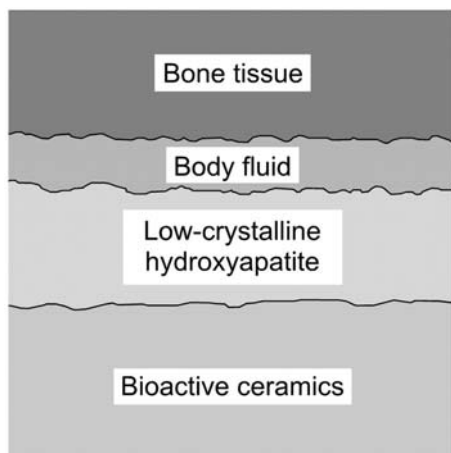
Figure 1. Bone-integrating mechanism of bioactive ceramics.

Bone-integrating ceramics, i.e. bioactive ceramics, has been developed for solving these problems. Hench *et al.* first discovered in early 1970's that some kinds of glasses in the system $Na_2O-CaO-SiO_2-P_2O_5$, afterward named Bioglass®, make direct bond to living bone without formation of the fibrous tissue around them (1). Since then, several kinds of bioactive ceramics, such as sintered hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (2,3) and glass-ceramic A-W containing crystalline oxyfluorapatite and wollastonite (4), have been developed during the last three decades. The bioactive ceramics have played an important role in bone repair in the fields of orthopedic surgery, neurosurgery and dentistry.

However load-bearing portions such as femur, tibia and tooth root cannot be replaced even by the bioacitve ceramics due to their insufficient mechanical properties. When implanted in such portions, brittle fracture is liable to occur due to lower fracture toughness than natural bone. In addition, stress shielding due to higher Young's modulus than the natural bone can consequently suppress growth of the surrounding bone. A lot of medical doctors require development of novel flexible bioactive materials, since they can be fabricated into desirable shapes according to condition of the bone defects. Ability of easy shaping of the natural bone is main reason why the bone transplantation is still popularly selected in medical treatments.

Natural bone is a kind of organic-inorganic composite fabricated from organic collagen and inorganic hydroxyapatite (5). Learning from this specific structure, design of organic-inorganic will provide an idea for development of novel bioactive materials able to solve problems of the bioactive ceramics.

3. MECHANISM OF BONE INTEGRATION

According to the previous reports on observation of the interface between the bioactive ceramics and the bone *in vivo*, they integrate with living bone through a hydroxyapatite layer which is formed at the interface by

chemical reaction with body fluid as shown in Figure 1 (6,7). Exceptionally tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) ceramics exhibit bioactivity without formation of such an hydroxyapatite layer (8). This hydroxyapatite layer is composed of carbonate hydroxyapatite with similar structure and composition to those of natural bone. Therefore, it is expected that fibroblastic cells could not proliferate on the hydroxyapatite layer, to result in direct contact of the bone with the surface of the materials, without intervention by the fibrous tissue. When this phenomenon occurs, tight chemical bond might be formed between the surface hydroxyapatite and the bone hydroxyapatite in order to decrease the interface energy between them. The bone-integrating ability therefore depends on the rate of the hydroxyapatite formation when the materials are exposed to body environment.

The hydroxyapatite formation on the bioactive ceramics and glass-ceramics can be well reproduced even in a simulated body fluid (SBF) that has almost similar concentration of inorganic ions to human blood plasma, as shown in Figure 2 (9,10,11,12). The hydroxyapatite formation is caused by chemical reaction between their surface and surrounding fluid, because SBF does not contain any cells and proteins. Materials able to form the hydroxyapatite layer on their surfaces in SBF have a potential to bond to living bone. This fluid can be used for not only evaluation of bioactivity of artificial materials *in vitro*, but also coating of hydroxyapatite on various materials under biomimetic conditions.

Although SBF can well reproduce chemical reaction of materials with body fluid *in vitro*, there is still a gap in composition between SBF and human extracellular fluid. Namely, SBF contains larger amount of Cl^- ion and smaller amount of HCO_3^- ion. Therefore the hydroxyapatite formed in SBF also contains larger amount of Cl^- ions and smaller amount of HCO_3^- than natural bone. Novel simulated body fluid with inorganic ion concentrations exactly equal to those of human extracellular fluid has been proposed by revising protocol of preparation including kind and amount of chemical reagents (12,13).

What kind of surface structure is effective for inducing bioactivity? In order to clarify this point, hydroxyapatite deposition behavior on various materials such as oxide gels prepared by sol-gel process, self-assembled monolayers (SAMs), chemically-treated metal substrates and so on was investigated in SBF. As a result, it has been revealed that various functional groups such as Si-OH (11,14), Ti-OH (14,15,16), Zr-OH (17,18), Ta-OH (19,20), Nb-OH (21), Mo-OH (22), COOH (23,24,25), PO_4H_2 (23) and SO_3H (26,27,28) and so on are effective for triggering heterogeneous nucleation of the hydroxyapatite. On the other hand, Al-OH groups do not induce the hydroxyapatite deposition (14). These findings bring us an idea that various bioactive organic-inorganic hybrids can be designed by organic modification of inorganic substances that provide the functional groups described above
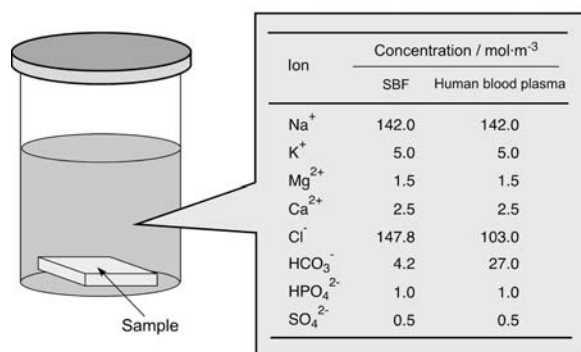
Figure 2. Ion concentration of simulated body fluid (SBF).

4. BIOACTIVE CERAMICS-DISPERSED COMPOSITES

Bioactive organic-inorganic composites have been popularly fabricated by mechanical mixing organic polymer and powders of bioactive ceramics. Their mixing ratio significantly affects mechanical property and biological affinity of the obtained composites. The bioactivity of the composite is mainly attributed to surface reactivity of the added ceramics. Therefore their bioactivity has tendency to be enhanced with increase in ceramic content. However addition of too much ceramics makes the composites hard and brittle. Optimization of the composition is quite important taking into account these points. Both bioresorbable and non-resorbable polymer can be used as an organic component according to applied parts.

4.1. Polyethylene-based composite

Ultra high molecular weight polyethylene (UHMWPE) has molecular weight ranging from 1,000,000 to 7,000,000 by polymerization using Ziegler-Natta catalyst. It is subjected to biomedical applications such as acetabular cup of artificial hip joint due to its high mechanical strength and wear resistance. Bonfield *et al.* developed polyethylene-hydroxyapatite composites by injection molding technique (29,30). Mechanical property and bioactivity are governed by volume content of hydroxyapatite. The composites exhibited sufficient flexibility at 40 vol% or less, while it became brittle at 45 vol% or more. The composite exhibits bioactivity at 20 vol% or more. Judging from these results, composites with hydroxyapatite content of 40 vol% have been commercialized. They are named HAPEX® and clinically used for artificial auditory ossicles.

Polyethylene-based composites containing other bioactive ceramics such as Bioglass® and glass-ceramics A-W have been also developed (31,32,33). In some composition, bending strength rather decreased with increase in ceramic content. This problem can be solved by formation of chemical bond between the ceramic powder and the polymer matrix using silane coupling agents. It is known that titania exhibits hydroxyapatite-forming ability in SBF (16). Hashimoto *et al.* developed polyethylene-titania composites (34). Those containing titania at 50 vol% exhibited bending strength of 68 MPa and Young's modulus of 7 GPa. The composites with titania content larger than 40 vol% formed the hydroxyapatite in SBF within 14 days. The bending strength of the composites was improved by silane coupling treatment of the titania powder (35).

4.2. Bioresorbable polymer-based composite

Poly-L-lactic acid (PLLA) is known as a bioresorbable polymer with high initial strength and is clinically used for bone fixation in the form of plates, screws and pins. Shikinami *et al.* synthesized PLLA-hydroxyapatite composites as follows (36). Low-crystalline hydroxyapatite particles were prepared from calcium hydrogen phosphate and calcium carbonate at 90°C. The obtained particles and PLLA were thermally kneaded to make the composites. The obtained composites exhibited high bending strength about 200 MPa. Moreover, addition of the hydroxyapatite particles to the composites even at 30-40 vol% did not decrease bending strength by forging at crystallizing temperature of PLLA. The composites formed the hydroxyapatite layer in SBF within 3 days. This is named Super FIXSORB® and commercialized by Takiron Co. Ltd., Japan.

Kikuchi *et al.* attempted preparation of the composites from beta-TCP and copolymer of PLLA and aliphatic polyester (37). FT-IR spectra of the composites confirmed that a chemical bond is constructed between carbonyl groups and Ca^{2+} in beta-TCP. The composites showed bending strength of 54 MPa. Takamatsu *et al.* attempted improvement of mechanical property of the beta-TCP/PLLA composites by ball-milling (38). PLLA solution and beta-TCP powder were mixed, ball-milled for various periods, and hot-pressed to obtain the composites. The bending strength increased with increase in ball-milling time. In particular, the composites prepared from PLLA with molecular weight of 120,000 by ball-milling for 48 hours exhibited bending strength of 80 MPa. SEM observation confirmed decrease in average particle size of beta-TCP with increase in ball-milling time. Suppression of aggregation of beta-TCP by the ball-milling contributed to enhancement of the bending strength.

Natural polysaccharides with high biological affinity are also available for component of the bioactive organic-inorganic composites. Several researchers have developed chitosan-hydroxyapatite composites through different techniques. Yamaguchi *et al.* used co-precipitation of chitosan and hydroxyapatite by addition calcium hydroxide to aqueous solution containing chitosan and phosphoric acid (39). On the other hand, Yoshida *et al.* attempted ball-milling of calcium phosphate particles as a precursor of the hydroxyapatite and chitosan sol to enhance homogeneity (40). Cellulose-hydroxyapatite composites can be also obtained by the ball-milling (41). However, the bending strength of most polysaccharide-based composites is as low as 5 to 10 MPa. To improve the mechanical strength, blending of thermoplastic polymer is attempted. Reis *et al.* revealed that the composites prepared from hydroxyapatite and polymer blend of starch and ethylene-
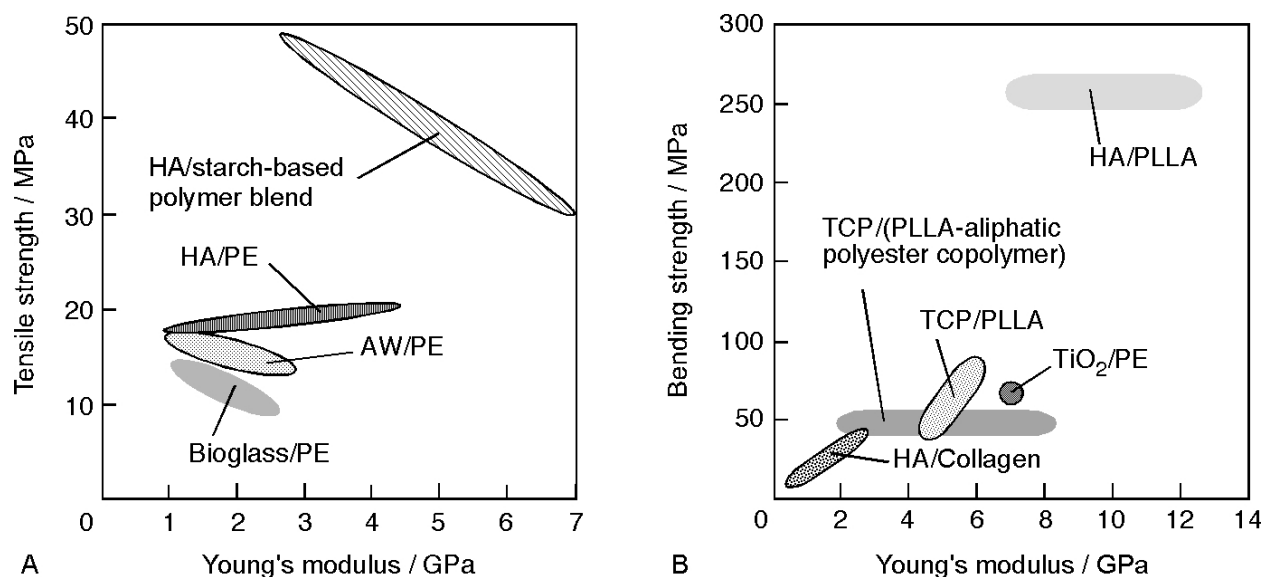
Figure 3. Tensile (A) and bending (B) strength of various ceramic-dispersed composites.

vinyl alcohol copolymer exhibited tensile strength more than 30 MPa (42).

Collagen-hydroxyapatite composite is expected to have high bioactivity due to its similar component to natural bone. Composites containing fibrous type I collagen and granule of hydroxyapatite and beta-TCP are commercialized in the name of Collagraft®. Kikuchi *et al.* prepared collagen-hydroxyapatite composites by co-precipitation process (43). Collagen and low-crystalline hydroxyapatite were co-precipitated by addition of calcium hydroxide to aqueous solution of the collagen and the phosphoric acid with keeping the solution neutral or weak alkaline condition. The obtained precipitate was pressed to fabricate the composites. The composites exhibited self-assembly where c axis of the hydroxyapatite was aligned parallel to the direction of collagen fibers. Effects of co-precipitation condition on ability of self-assembly were investigated. As a result, the self-assembly was most remarkably observed at 40°C at pH 8 to 9. Isoelectric point of collagen is reported to be pH 8 to 9. Significant collagen precipitation near the isoelectric point is considered to enhance the self-assembly. The highly self-assembled composite exhibited high bending strength. Mechanical properties of various ceramics-dispersed organic-inorganic composites are summarized in Figure 3.

5. ORGANIC-INORGANIC NANOCOMPOSITE

As described above, popular fabrication technique of the organic-inorganic composite is mechanical blending of the ceramic powder and the organic polymer. However homogeneity at nanometer and molecular level is difficult to be achieved in this process. On the contrary, sol-gel process enables homogenization at the molecular level. In this process, metal alkoxide is mixed with organic polymer and then subjected to hydrolysis and polycondensation of the alkoxide. As a result, inorganic domains with nanometer size are dispersed in the polymer

matrix of the composites. It has been already reported that organically modified silicates can be synthesized from tetraethoxysilane (TEOS, $Si (OC_2H_5)_4$) with incorporation of poly (dimethylsiloxane) (PDMS) by Mackenzie *et al.* The synthesized organic-inorganic composite, called ORMOSILS, may have properties of thermal stability from silica block as well as flexibility from polymer chain (44).

Osaka *et al.* paid attention to the fact that ORMOSILS contain a lot of Si-OH groups and reported that incorporation of calcium nitrate in ORMOSILS during the sol-gel processing gave the composites hydroxyapatite-forming ability in SBF (45). Si-OH groups and Ca^{2+} ions on ORMOSILS trigger nucleation of the hydroxyapatite in SBF. This means that the OROMSILS incorporated with calcium salt shows bone-bonding property when implanted in the body. Chen *et al.* modified protocol of the synthesis and obtained TEOS-PDMS-$Ca(NO_3)_2$ composites with higher mechanical strength (46).

On the application, such bioactive organic-inorganic composites must have various functionalities including mechanical properties, workability and so on, through their modification with organic polymers. The present authors reported the potential on providing organic substances with the bone-bonding properties through modification of organic-inorganic composites by incorporation of methacryloxypropyltrimethoxysilane (MPS, $CH_2=C(CH_3)COO(CH_2)_3Si(OCH_3)_3$) and calcium salt into 2-hydroxyethylmethacrylate (HEMA, $CH_2=C(CH_3)COOCH_2CH_2OH$) (47). HEMA has high hydrophilicity and high biological affinity, and is used for medical applications as contact lens and coating agent on artificial blood vessels (48). MPS has alkoxysilane group that may provide Si-OH groups after hydrolysis, whereas HEMA may provide hydrophilic polymer matrix in the composite. Polymers from MPS and HEMA were synthesized at several compositions, and then the polymer solution was mixed with calcium chloride ($CaCl_2$).

Figure 4. Flexible MPS-HEMA nanocomposite.
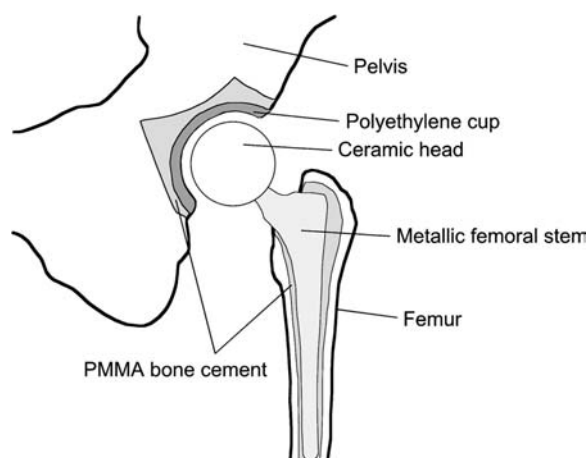
Figure 5. Fixation of artificial hip joint with PMMA bone cement.

Mixture of MPS and HEMA was dissolved in ethanol and polymerized by heat treatment at 75°C with benzoylperoxide (BPO) as initiator. Then ethanol solution of $CaCl_2$ was added and dried for 2 weeks at room temperature to form a bulk gel. The obtained composites exhibited as high flexibility as they can be easily bent bay hand (Figure 4). They formed the hydroxyapatite in SBF at the compositions of MPS:HEMA=0.1:0.9. This means that organic-inorganic nanocomposites obtained by the sol-gel process can exhibit bioactivity even at small amount of inorganic component

Bioactive organic-inorganic composites can be obtained from various polymers such as chitin (49), chitosan (50), starch (51), polycaprolactone (52) and polyvinyl alcohol (PVA) (53) through the sol-gel process. These mean that bioresorbability of the composites in body environment can be easily controlled. In addition, such composite can be fabricated by addition of tetraisopropyl titanate (Ti $(OCH(CH_3)_2)_4)$ to HEMA (54).

6. BIOACTIVE BONE CEMENT UTILIZING ORGANIC-INORGANIC COMPOSITE

Material design based on the organic-inorganic composite is quite effective for providing polymethyl methacrytlate (PMMA) bone cement with bioactivity. This type of cement is composed of powder containing PMMA and liquid containing methyl methacrytlate (MMA). The paste obtained by mixing the powder and the liquid is injected into the bone defect and polymerized for setting (Figure 5) (55). This type of cement is clinically used for fixation of artificial joints and reconstruction of vertebra. However lack of the bioactivity of the cements may cause loosening at the interface between the bone and the injected cement. In some severe cases, the implanted artificial joints must be extracted and replaced by new ones. There are two techniques for preparation of the bioactive PMMA bone cements, (1) addition of the bioactive ceramics to the powder, and (2) modification of the cements by bioactive inorganic substances at molecular level.

As technique (1), Moroni *et al.* investigated effects of addition of the hydroxyapatite to the PMMA cement on properties of the cements (56). It was confirmed that creep resistance of the cement was improved by addition of the hydroxyapatite at 5 mass%, and that heat generation due to polymerization of MMA was suppressed. Shinzato *et al.* reported effects of various bioactive ceramics such as the hydroxyapatite, glass-ceramics A-W and $MgO-CaO-SiO_2-P_2O_5-CaF_2$ glass (mother glass of the glass-ceramics A-W) (57). Each bioactive ceramic powder was added at 70 mass% to the cement. Bone integration was observed for all the modified cements. In particular, degree of the bone integration was the highest in the $MgO-CaO-SiO_2-P_2O_5-CaF_2$ glass. This phenomenon was attributed to quite high reaction rate of the glass with body fluid.

Goto *et al.* modified the PMMA cement with titania nanoparticles (58). When the titania was added at 50 mass% to the cement, the modified cement exhibited bone integration. In addition, the bioactivity of the cement was improved by previous silane coupling treatment of the titania.

As technique (2), the present authors attempted chemical modification of the PMMA cement with Si-OH group and Ca^{2+} ions. The modified cement was prepared from the powder added with various calcium salts such as $CaCl_2$, $Ca(CH_3COO)_2$, $Ca(OH)_2$ and $CaCO_3$, as well as the liquid added with MPS (59). Content of the additive was 20 mass% to the cement. The cements modified with $CaCl_2$, $Ca(CH_3COO)_2$, $Ca(OH)_2$ formed the hydroxyapatite in SBF, but not those unmodified and modified with $CaCO_3$. Solubility of the calcium salts changes in the order: $CaCl_2 > Ca(CH_3COO)_2 > Ca(OH)_2 > CaCO_3$. This means that release of Ca^{2+} ion from the cement to the surrounding fluid is important for exhibiting bioactivity. It is noted that content of the inorganic components necessary for achieving bioactivity is lower than the technique (1). Therefore handling properties of the original PMMA can be well maintained.

7. CONCLUSION

Various bone-integrating organic-inorganic composites have been developed from hybridization of bioactive ceramics and organic polymer at micrometer or nanometer level. Some of them have already subjected to clinical applications. It is expected that development of the composites is further progressed, and that more and more artificial bone substitutes are used in medical fields.

8. ACKNOWLEDGMENTS

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