Development of bioactive materials based on bone-bonding mechanism on metal oxides

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Bioactive ceramics have high biological affinity that enables direct bone-bonding. However they do not have mechanical performances enough to repair bone tissue under loaded conditions. It has been revealed that most materials exhibiting bioactivity form bone-like apatite in body environment. We investigated apatite-forming ability of tantalum- and niobium-based oxide gels in simulated body environment in order to fundamentally clarify chemical structure effective for achieving bioactivity. We found that Ta-OH and Nb-OH groups on the surfaces of the oxide gels have a potential to form the apatite. On the basis of these findings, we have successfully provided tough tantalum metal with bioactivity by simple NaOH and heat treatments that form a lot of Ta-OH groups on their surfaces. We also synthesized bioactive organic-inorganic hybrids with flexibility by organic modification of inorganic chemical species with apatite-forming ability. A sequence of material design described above would produce bioactive materials with various mechanical properties.

Key-words : Bioactivity, Apatite, Simulated body fluid (SBF), Metal oxide, Functional group, Bioactive metal, Bioactive organic-inorganic hybrid, Bioactive bone cement, Bone substitutes

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1. Introduction

Bone defects can be sometimes repaired by own bones extracted from other parts of the patient. However available bone tissue is limited to the only small amounts. Therefore artificial materials to repair the bone defects are needed. However artificial materials implanted into the bone defects are generally encapsulated with a fibrous tissue of collagen. This is a normal reaction for protecting our living body from foreign substances. The implanted material is consequently isolated from the surrounding bone and does not bond to the living bone.

On the other hand, several kinds of ceramics such as $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2-\text{P}_2\text{O}_5$ glasses named Bioglass[®],^{1,2)} sintered hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$),³⁾ glass-ceramic A-W^{4,5)} containing crystalline apatite and wollastonite and so on are found to directly bond to living bone. They are called bioactive ceramics, meaning that they elicit and modulate specific biological activity.⁶⁾ Bone-bonding property of the bioactive ceramics is a quite attractive feature for bone reconstruction, since tight fixation to the surrounding bone can be achieved. However their problem is that they have lower fracture toughness than those of human cortical bone.⁷⁾ Therefore they cannot be used in highly load-bearing portions such as femoral and tibial bones. Novel design of tough bioactive materials is needed.

It is known that most bioactive ceramics achieve the bone-bonding through the formation of low-crystalline apatite layer on their surfaces as shown in Fig. 1.^{8,9)} This type of apatite has similar composition and structure to natural bone and is often called "Bone-like apatite". Osteoblast can proliferate in preference to fibroblast on the formed apatite, thereby leading to production of bone matrix including col-

Fig. 1. Bone-bonding mechanism of bioactive ceramics.

lagen fiber and apatite. As a result, the surrounding bone comes into direct contact with the surface apatite on the bioactive material. Afterwards tight chemical bond is produced between the material and the surrounding bone. Consequently the essential requirement for an artificial material to exhibit bioactivity is the formation of bone-like apatite layer on its surface in body environment. The same type of the apatite is formed on the bioactive materials even in an acellular simulated body fluid (SBF) with inorganic ion concentrations nearly equal to those of human extracellular fluid, which was proposed by Kokubo et al.¹⁰⁻¹²⁾ Bioactivity of an artificial material can be well evaluated *in vitro* by examining formation of the apatite layer on its

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surface in SBF.¹³⁾

The key issue for the apatite formation on an artificial material is functional groups abundant on its surface, since the body fluid is already supersaturated with respect to the apatite even under the normal conditions. Fundamental researches have been performed in order to reveal what kind of functional group is effective for the apatite formation in body environment by using sol-gel derived oxide gels, self-assembled monolayer (SAM) and so on. Several functional groups such as Si-OH,^{11),14)} Ti-OH,^{14),15)} Zr-OH,¹⁶⁾ COOH,¹⁷⁾ PO₃H₂,¹⁷⁾ SO₃H^{18),19)} have been found to possess the apatite-forming ability, but Al-OH group does not. In addition, release of Ca²⁺ ions from the materials significantly accelerates the apatite nucleation by increasing degree of supersaturation of the surrounding fluid with respect to the apatite.²⁰⁾

In this study, we review design of bioactive materials with various mechanical performances based on the apatite formation on oxides induced by functional groups, including our research outcomes.

2. Design of bioactive materials based on tantalum and niobium

We especially paid our attention on tantalum- and niobium-based materials. Tantalum and niobium metals have high workability due to their excellent malleability and ductility. In addition, they have been known as elements with lower cytotoxicity than titanium.^{21),22)} Therefore they are clinically used for components of vanadium-free titanium alloys for hip joints.²³⁾ Recently carbon porous bodies coated with tantalum have been subjected to clinical use as artificial patella and acetabular cup.^{24),25)}

We synthesized tantalum oxide and niobium oxide hydrogels through sol-gel process and examined their apatite formation in SBF. Pure tantalum oxide and niobium oxide gels formed the apatite on their surfaces in SBF within 7 d as shown in Fig. 2.^{26),27)} This means that Ta-OH and Nb-OH groups are also effective for the heterogeneous apatite nucleation in body environment. The apatite-forming ability of the tantalum oxide gels was enhanced by incorporation with Na⁺ ions to form sodium tantalate. The incorporated Na⁺ exchanges with H₃O⁺ in SBF to form a lot of Ta-OH groups. In addition, increase in pH due to the ion exchange increases degree of supersaturation with respect to the apatite.

3. Preparation of bioactive metals by surface modification

On the basis of the studies concerning the apatite deposition on the various metal oxide gels, tough metallic materials can be provided with bioactivity through surface modification to form the functional groups with the apatite-forming ability.

Kim et al. proposed simple NaOH and heat treatments to prepare bioactive titanium metal and its alloys.²⁸⁾⁻³¹⁾ The metals formed amorphous sodium titanate on their surfaces by the treatment and the apatite after subsequent soaking in SBF. Direct bone-bonding was observed when the treated metals were implanted into rabbit tibial bone.³²⁾ Titanium metals can be provided with bioactivity by another chemical modification using hydrogen peroxide (H₂O₂) solution added with tantalum chloride (TaCl₅).³³⁾⁻³⁵⁾

We applied the NaOH and heat treatments to the tantalum metal.^{36),37)} Even untreated tantalum metal formed the

Fig. 2. SEM photographs of the surfaces of tantalum oxide and niobium oxide gels after soaking in SBF for 7 d.

Fig. 3. SEM-EDX profiles of the surface (left) and cross-section (right) of NaOH- and heat-treated tantalum metals after soaking in SBF for 28 d.

apatite on its surface in SBF after 28 d. However this induction period is too long for practical use. When the metal was treated with 0.5 kmol·m⁻³ NaOH aqueous solution at 60°C for 24 h and heated at 300°C for 1 h, amorphous sodium tantalate was formed on its surface. It was confirmed by Raman spectroscopy that the sodium tantalate contained chemical structure composed of TaO₆ octahedra distorted by Na⁺ similar to the sol-gel-derived sodium tantalate. The treated metal formed the apatite on its surface within 4 d in SBF. The formed apatite layer is shown in Fig. 3.

Adhesive strength of the apatite layer formed on the tantalum metals in SBF to the substrates was quantitatively measured by tensile test.³⁸⁾ The apatite layer formed on the NaOH and heat-treated tantalum metal showed higher bonding strength (14.79 ± 2.74 MPa) than that formed on the untreated metal (9.08 ± 1.38 MPa). In order to clarify the difference, we precisely characterized surface structure of the tantalum metals by using Auger electron spectroscopy (AES). The measured AES profiles are shown in Fig. 4. The untreated metals steeply decreased O concentration and steeply increased Ta concentration with increasing depth from the top surface. On the other hand, NaOH and heat-treated metals gradually decreased O concentration and gradually increased Ta and Na concentrations. This means that the surfaces of the treated metals have a highly graded structure. Such a graded structure maintained even after the apatite deposition in SBF. Enhancement of bonding strength by the NaOH and heat treatments is attributed to the graded structure produced by the treatments.

When the treated metals are implanted in rabbit tibia, they directly bonded to the living bone through the apatite layer formed in the body as shown in Fig. 5.³⁹⁾ These results indicate that the results on model experiments using sodium

Fig. 4. AES depth profiles of the surfaces of untreated (left) and NaOH- and heat-treated (right) tantalum metals as a function of depth from the top surface.

Fig. 5. SEM-EDX profile (left) and contact radio micrograph (CMR, right) of the cross-section of NaOH- and heat-treated tantalum metals after implantation in rabbit tibia for 16 weeks.

tantalate hydrogels well predict chemical structure effective for achieving bioactivity.

4. Preparation of bioactive organic-inorganic hybrids

Even metallic materials have problems to be solved for bone reconstruction. Namely, their Young's modulus is higher than those of human cortical bone. This is liable to prevent load transfer to the surrounding bone and consequent bone regeneration. The phenomena are called stress-shielding. Therefore novel bioactive materials with not only fracture toughness but also Young's modulus analogous to natural bone are needed as ideal bone substitutes. It is known that natural bone is a kind of organic-inorganic hybrid where organic collagen and inorganic apatite crystals are three-dimensionally woven at nanometer level.⁴⁰⁾ These facts bring us an idea that organic-inorganic hybrids where bioactive chemical species are modified with organic polymers can be a candidate for novel bone-repairing materials described above.

Mackenzie et al. reported that organically modified silicates (ORMOSILS) can be synthesized from tetraethylorthosilicate (TEOS) by incorporation of poly (dimethylsiloxane) (PDMS).⁴¹⁾ Tsuru et al. revealed that incorporation of calcium nitrate into the ORMOSILS during the sol-gel processing gave the hybrids apatite-forming ability in SBF.⁴²⁾ Si-OH groups and Ca^{2+} ions on ORMOSILS trigger nucleation of the apatite in SBF. Chen et al. modified protocol of the synthesis and obtained TEOS-PDMS- $\text{Ca}(\text{NO}_3)_2$ hybrids with higher mechanical strength.⁴³⁾

We attempted synthesis of bioactive organic-inorganic

Fig. 6. SEM photographs of the surfaces of MPS-HEMA hybrids before and after soaking in SBF for 7 d.

hybrids by incorporation of methacryloxypropyltrimethoxysilane (MPS, $\text{CH}_2=\text{C}(\text{CH}_3)\text{COO}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$) and calcium chloride (CaCl_2) into 2-hydroxyethylmethacrylate (HEMA, $\text{CH}_2=\text{C}(\text{CH}_3)\text{COO}(\text{CH}_2)_2\text{OH}$).^{44),45)} HEMA has high hydrophilicity and high biological affinity, and is used for medical applications as contact lens and coating agent on artificial blood vessels.⁴⁶⁾ MPS and HEMA were dissolved in ethanol and heated at 75°C for 3 h with benzoylperoxide as initiator for polymerization of HEMA and MPS. Then the obtained polymer solution was mixed with ethanol solution containing CaCl_2 . The resultant solutions were cast in polypropylene containers and dried at room temperature for 14 d. Composition of the hybrids was fixed at MPS : HEMA : $\text{CaCl}_2 = 1 : 9 : 1$ in molar ratio. The hybrids formed the apatite in SBF as shown in Fig. 6.

Various natural and synthetic polymers are available for synthesis of the hybrids. Recent researches have reported preparation of bioactive organic-inorganic hybrids from chitin,⁴⁷⁾ chitosan,⁴⁸⁾ starch,⁴⁹⁾ polycaprolactone⁵⁰⁾ and poly (vinyl alcohol) (PVA)⁵¹⁾ by modification with Si-OH and Ca^{2+} ions. We also found that this type of bioactive organic-inorganic hybrids can be prepared from tetraisopropyltitanate (TiPT) that provides Ti-OH group and HEMA by addition of CaCl_2 .⁵²⁾

Material design utilizing organic-inorganic hybrids can be also applied to preparation of bioactive polymethylmethacrylate (PMMA) bone cements. The cement is composed of PMMA powder added with small amount of polymerization initiator, and setting liquid made from methylmethacrylate (MMA) monomer.⁵³⁾ The powder and the liquid are mixed and injected into the gap between the artificial joints and the

Fig. 7. SEM photographs of the surfaces of PMMA cements which were modified with MPS and different calcium salts after soaking in SBF for 7 d. "Reference" means the cement without modification.

bone. This type of cement is widely used for fixation of the artificial hip joints in orthopedic fields. The problem with the cements is that they do not show bioactivity. Loosening between the bone and cement is liable to occur after a long implantation period.

We prepared novel PMMA cements by chemical modification with Si-OH and Ca^{2+} ions.^{44),54),55)} Namely, we added different calcium salts to the powder, and MPS to the liquid. The prepared cements after soaking in SBF are shown in Fig. 7. Those modified with CaCl_2 , $\text{Ca}(\text{CH}_3\text{COO})_2$ and $\text{Ca}(\text{OH})_2$ formed the apatite on their surfaces, but not that modified with CaCO_3 . Solubility of the used calcium salts in water increases in the order $\text{CaCO}_3 < \text{Ca}(\text{OH})_2 < \text{Ca}(\text{CH}_3\text{COO})_2 < \text{CaCl}_2$. Addition of calcium salts with high solubility would enhance the apatite formation by release of large amount of Ca^{2+} ions.

5. Conclusion

Bioactive materials with various mechanical properties can be designed through clarification of functional groups effective for apatite formation in body environment and subsequent surface modification of materials with such functional groups. On the basis of this type of material design, we have successfully provided various materials including metals, organic-inorganic hybrids and conventional bone cements with bioactivity.

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