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Development of bioactive materials based on surface chemistry

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Abstract

All bioactive materials developed up to 1990 were based on calcium phosphate. It was later revealed that materials that form bonelike apatite on their surfaces in the living body bond to living bone through the apatite layer, and that apatite formation on a material is induced by various functional groups on its surface. Based on these findings, bioactive titanium was prepared by forming sodium titanates on its surface via NaOH and heat treatments, and applied to an artificial total hip joint. Porous titanium metal able to exhibit osteoconductivity as well as osteoinductivity was prepared by forming anatase on its surface via NaOH, HCl and heat treatments. Various bioactive materials with different physical properties are expected to be derived from ceramics, metals and organic polymers by modifying their surfaces with functional groups effective for apatite nucleation.

Keywords: Interface; Apatite; TiO_2; Biomedical applications; Bioactivity

1. Introduction

Artificial materials implanted into bone defects are generally encapsulated by fibrous tissue and thus isolated from the surrounding bone. Materials for bone substitutes are desired to bond to living bone. As bone is mainly composed of calcium phosphate, the bone-bonding abilities of various calcium phosphates have been examined in animal experiments. After these trials, Hench et al. showed that some Na_2O-, and SiO_2-based glasses containing CaO and P_2O_5 spontaneously bond to living bone.[1] These glasses were the first man-made materials found to bond to living bone. Since then, various glasses containing CaO and P_2O_5,[2] glass–ceramics containing apatite[3,4] and sintered calcium phosphate ceramics such as hydroxyapatite,[5] β-tricalcium phosphate[6] and their combinations[7] were also found to bond to living bone and have been used clinically as bone substitutes. However, their mechanical strengths were lower than that of human cortical bone. Kokubo et al. developed glass–ceramic A–W, containing apatite and wollastonite.[8] This glass–ceramic showed a higher mechanical strength than that of the human cortical bone, as well as high bone-bonding ability, i.e. bioactivity. However, even glass–ceramic A–W was not able to be used under highly loaded conditions such as in the femur, as its fracture toughness was lower than that of human cortical bone.

Therefore, the development of bioactive materials with higher fracture toughness was desired. How was it possible? All bioactive materials developed up to 1990 were based on calcium phosphate. Is it an essential requirement for bioactive material to contain calcium phosphate?

2. What kind of material bonds to living bone?

Natural calcite ($CaCO_3$)[9] and $CaO–SiO_2$ glasses[10] were found to bond to living bone. These materials do not contain P_2O_5, but CaO. What is the requirement for bioactive materials?

When the interfaces between living bone and the bioactive materials developed up to 1990 were observed under the scanning electron microscope (SEM) and transmission electron microscope (TEM), it was found that most of the bioactive materials,[2,4,11–14] except β-tricalcium phosphate ceramics[15] and natural calcite,[16] were connected to the living bone through a non-collagenous apatite layer. This apatite layer was reproduced on their surfaces even in acellular simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma.[17]

According to detailed analysis of the surface layer, the surface apatite was found to be very similar to bone mineral in its composition and structure.[18] It was therefore expected that osteogenic cells preferentially proliferate and differentiate to produce bone tissue on these surfaces,[19] as occurs on fractured bone. As a result, new bone might grow from the surrounding bone and

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come into direct contact with the surface apatite.[20] When this occurs, a tight chemical bond could be formed between the bone mineral and the surface apatite,[21] again, as occurs in the healing of fracture bone.

From these findings, it can be said that materials that form bonelike apatite on their surfaces in the living body can bond to living bone through this apatite layer, and that the apatite-forming ability of a material can be evaluated in SBF, without the need for animal experiments. This can be a useful guideline in searching for new bioactive materials. Since then, material scientists could systematically investigate the bone-bonding abilities of materials as a function of their composition and structure according to this guideline. In 2007, the SBF was standardized as the solution for in vitro evaluation for apatite-forming ability of implant materials by the International Organization for Standardization (ISO 23317).

3. What kind of material forms bonelike apatite?

Now, our question regarding what type of material bonds to living bone moves to the question, what kind of material forms bonelike apatite? Various materials were examined for their apatite-forming abilities in SBF. Consequently, gels of SiO_2,[22] TiO_2,[23] ZrO_2,[24] Nb_2O_5[25] and Ta_2O_5[26] prepared by sol–gel methods were found to form apatite on their surfaces in SBF. This indicates that the SiOH, TiOH, ZrOH, NbOH and TaOH groups abundant on their surfaces are effective for apatite nucleation. Once the apatite nuclei are formed, they can grow spontaneously by consuming calcium and phosphate ions from the surrounding SBF, as SBF is supersaturated with respect to the apatite. In addition, by examining the apatite-forming ability of self-assembled monolayers terminating with various functional groups, the functional groups PO_4H_2 and COOH were also found to be effective for apatite nucleation.[27] It was also recently shown that sulfonic groups (SO_3H) incorporated onto organic polymers are effective for the apatite nucleation.[28]

These results indicate that various bioactive materials with different physical properties can be derived not only from CaO, P_2O_5-based ceramics, but also from CaO- and P_2O_5-free ceramics, and even from metals and organic polymers, if their surfaces

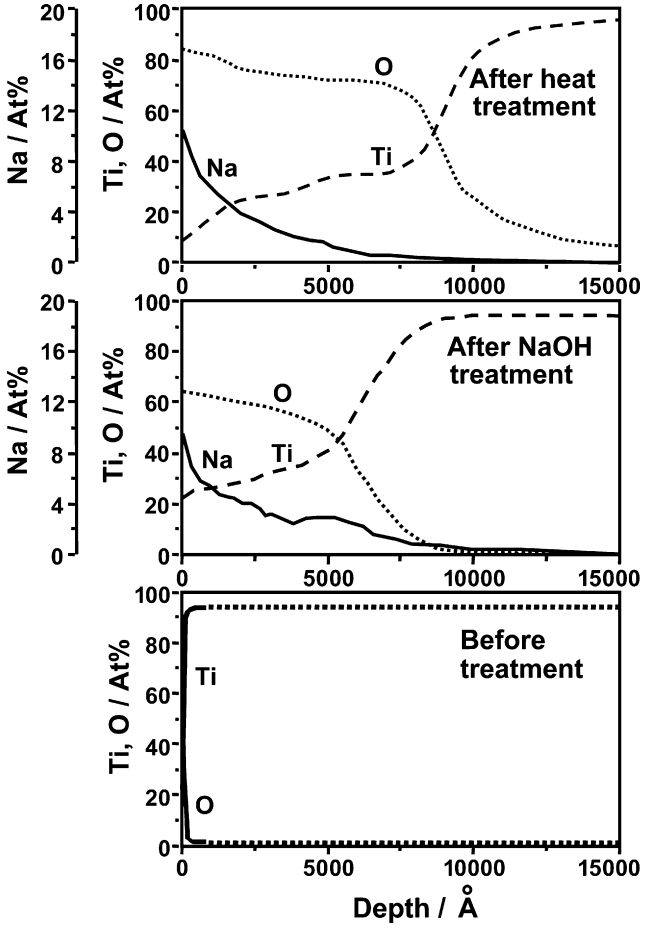

Fig. 1. Auger electron spectra profiles of surfaces of Ti metals before treatment, and after NaOH and heat treatments, as a function of depth.

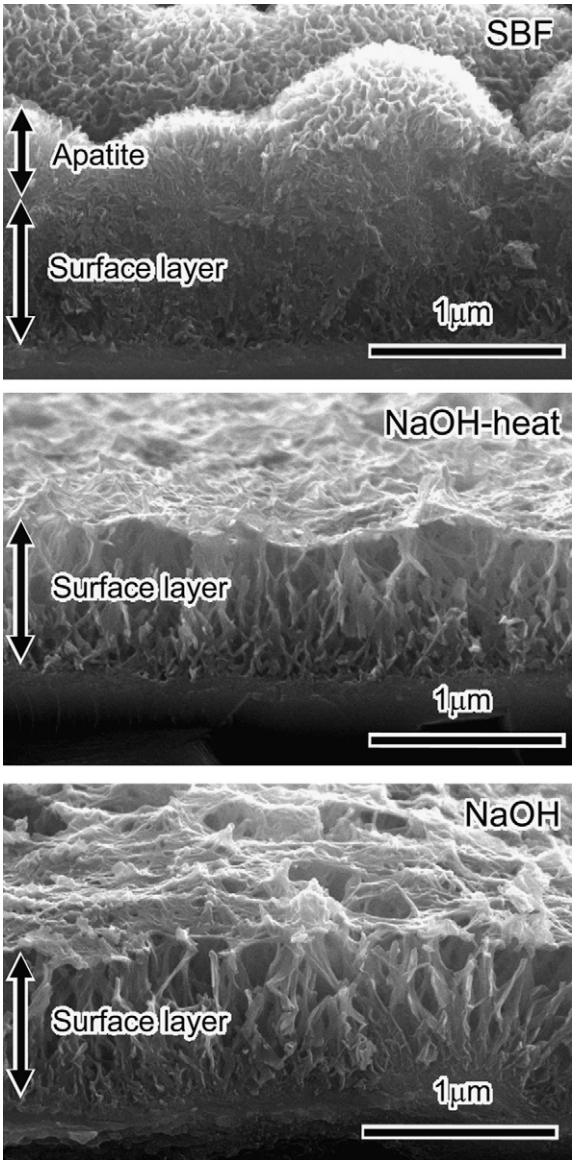

Fig. 2. SEM pictures of cross-sections of surfaces of Ti metals subjected to NaOH and heat treatments and subsequently soaked in SBF.

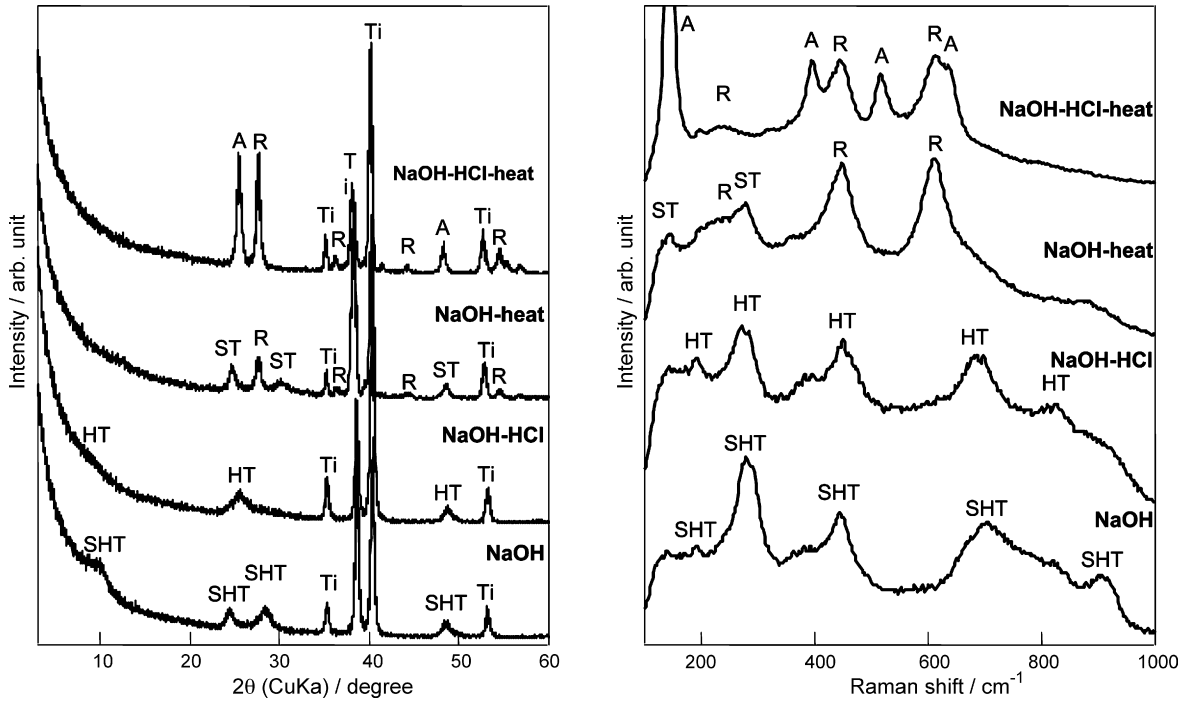

Fig. 3. TF-XRD patterns (left) and Raman spectra (right) of surfaces of Ti metals subjected to NaOH, HCl and heat treatments. Ti; α-Ti, SHT; Sodium hydrogen titanate, HT; Hydrogen titanate, ST; Sodium titanate, R; Rutile, A; Anatase.

are modified with functional groups effective for apatite nucleation.

4. Development of osteoconductive metals

It is well known that titanium metal and its alloys are generally covered with a thin TiO_2 passive layer. It is assumed that if Na_2O is incorporated into the surface TiO_2 layer, these metals could form many Ti–OH groups on their surfaces in the living body, since Na^+ ions could be released into the body fluid via exchange with H_3O^+ ions. As a result, they can be expected to form a bonelike apatite layer on their surfaces in the living body and bond to living bone through this apatite layer.

When titanium metal was soaked in a 5 M NaOH solution at 60 °C for 24 h, sodium and oxygen penetrated up to 1 μm into the surface of the titanium metal, with the content gradually decreasing with increasing depth, giving a gradient structure, as shown in Fig. 1.[29] When it was subsequently heat treated at 600 °C for 1 h, only oxygen penetrated deeper into the metal, without disturbing the gradient structure.

A layer of a lathlike structure was found to be formed on the surface of titanium metal by the NaOH treatment, and this became slightly denser with the subsequent heat treatment, as shown in Fig. 2. The scratch resistance of the surface layer was also increased from 3 to 50 mN by the heat treatment.

According to thin-film X-ray diffraction (TF-XRD) and Raman spectroscopy, the lathlike layer formed by the NaOH treatment consisted of sodium hydrogen titanates ($Na_xH_{2x}Ti_yO_{2y+1}$; $0<x<2$ and $y=2$, 3 or 4), which were transformed into sodium titanates ($Na_2Ti_yO_{2y+1}$; $y=5$, 6, etc.) and rutile by the subsequent heat treatment, as shown in Fig. 3.

When the titanium metal subjected to the NaOH treatment was soaked in SBF, it formed apatite on its surface as expected, as shown in Fig. 4.[30] The apatite-forming ability of the titanium metal was much increased by the subsequent heat treatment, because of the stabilization of the sodium titanate.[31] On the NaOH- and heat-treated titanium metal, the apatite was found to start to precipitate in the interior of the lathlike layer, fill the interspace of the lathlike phases integrating with them, and then grow over the surface of the lathlike layer, as shown in Fig. 2. The thus formed apatite was confirmed from TEM images and EDX spectroscopy (Fig. 5) to have a nanometre-sized needlelike form and have a composition with a Ca/P atomic ratio of 1.65 with small amounts of Na and Mg, similar to bone mineral.[32]

According to TEM observations,[32] X-ray photoelectron spectroscopy[33] and zeta potential measurement,[34] the mechanism of apatite formation on the NaOH- and heat-treated titanium metal in SBF was interpreted in terms of electrostatic interactions between the surface of the titanium metal and the ions in the SBF, as shown in Fig. 6. The sodium titanate at the surface releases Na^+ ions via exchange with H_3O^+ ions in the SBF to form many Ti–OH groups on the surface. As a result, the surface is negatively charged and reacts with the positively charged Ca^{2+} ions in the SBF to form calcium titanate. As the Ca^{2+} ions accumulate, the surface becomes positively charged and reacts with negatively charged phosphate ions to form amorphous calcium phosphate. This calcium phosphate is metastable and hence eventually transforms into stable crystalline bonelike apatite.

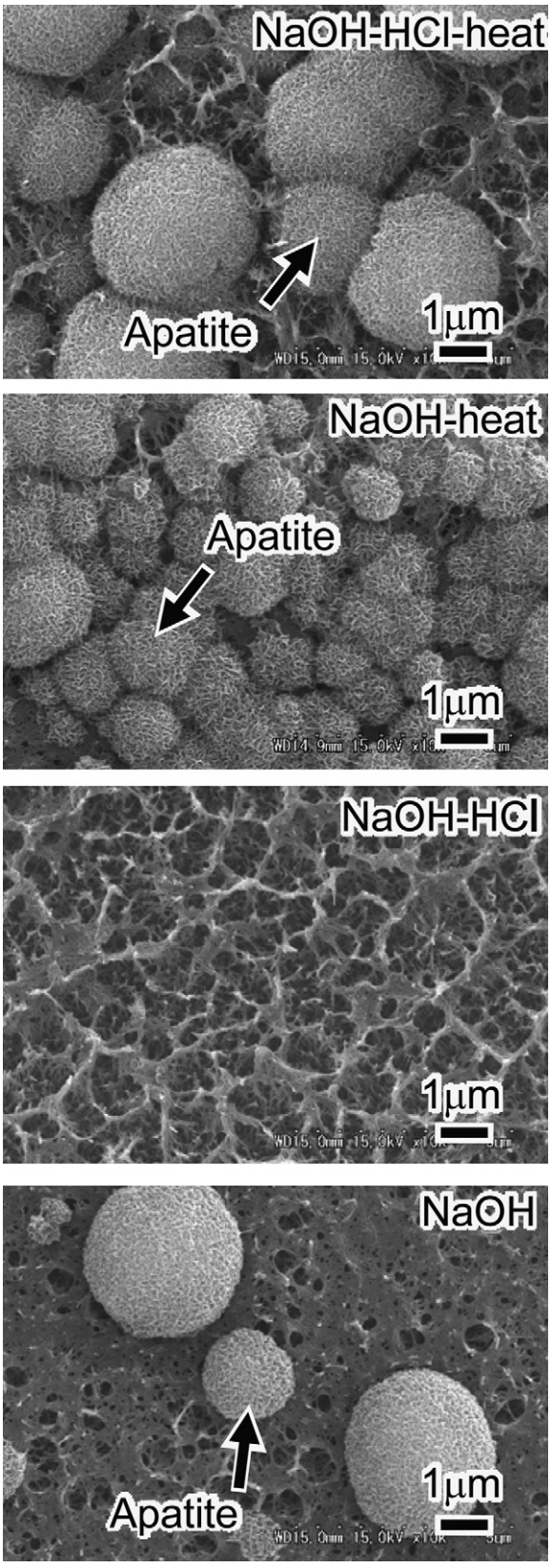

Fig. 4. SEM pictures of surfaces of Ti metals subjected to NaOH, HCl and heat treatments, and soaked in SBF for 1 day.

Thus, treated titanium metal is expected to form bonelike apatite on its surface in the living body and bond to living bone through the apatite layer. A titanium metal rod subjected to the same treatments was implanted into the intramedullary canals of rabbit femurs. It was found that an apatite layer formed on the surface of the NaOH- and heat-treated titanium metal within 3 weeks, as expected, and the metal was tightly bonded to the surrounding bone within 12 weeks, as shown in Fig. 7.[35] At 12 weeks after the implantation, the rod could not be pulled from the intramedullary canal without being accompanied by bone fragments, as shown in Fig. 8.

These NaOH and heat treatments were applied to a porous titanium layer on a prosthetic hip joint made of a titanium alloy, as shown in Fig. 9, and subjected to clinical trial in 70 patients. The hip joints were fully covered with newly grown bone and tightly fixed to the surrounding bone. The Ministry of Health, Labour and Welfare, Japan, approved this prosthesis for sale in August 2007.

5. Development of osteoinductive metals

According to the results of apatite formation on titania gels, even Na_2O-free titanium oxide can be expected to form apatite on its surface in the body environment. When NaOH-treated titanium metal was soaked in 0.5 mM HCl solution at 40 °C for 24 h, the sodium hydrogen titanates at the surface released all their Na^+ ions and were converted to hydrogen titanates ($H_2Ti_yO_{2y+1}$; $y = 2$, 3 or 4), as shown in Fig. 3. As a result, the apatite-forming ability of the surface of the titanium metal was lost, as shown in Fig. 4. However, when the NaOH- and HCl-treated titanium metal was heat-treated at 600 °C for 1 h, the hydrogen titanates at the surface was converted to anatase and rutile, as shown in Fig. 3 and gave high apatite-forming ability, almost equal to that of the NaOH- and heat-treated titanium metal, as shown in Fig. 4. This increase in apatite-forming ability following heat treatment is not attributed to the increase in the number of Ti–OH groups on the surface of the titanium metal, but to different factors that are the subject of future research. Anyway, the mechanism of apatite-formation on this titanium oxide also could be interpreted in terms of surface chemistry.

Porous titanium metal with 40 vol% of connected pores 300–500 μm in size, prepared by plasma spraying of titanium granules, was modified by the NaOH, HCl and heat treatments to give titanium oxide on the surfaces of the pore walls. When implanted into defects in the femurs of rabbits, it was penetrated by bone grown from the surrounding bone and was tightly bonded to the bone.[36] In addition to bone defects, this porous titanium metal formed bony tissue from the centre to the periphery when implanted in the muscles of beagle dogs, as shown in Fig. 10.[37] This type of bioactive material, able to exhibit osteoconductivity as well as osteoinductivity, is believed to be useful as bone substitutes such as artificial vertebrae, especially in large bone defects, as bone is formed from the central part of the implant as well as from the periphery. Porous calcium phosphate ceramics are also reported to exhibit osteoconductivity as well as osteoinductivity.[38] In comparison with them, porous titanium metals show higher mechanical strength.

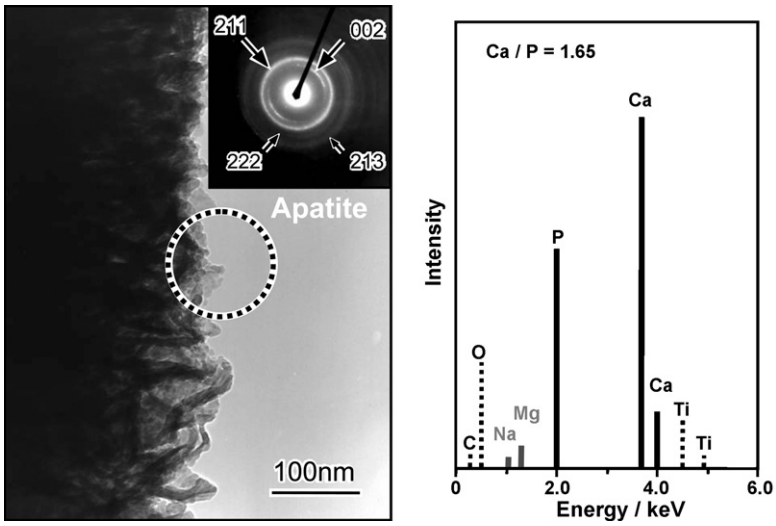

Fig. 5. TEM picture and EDX spectrum of surface of NaOH- and heat-treated titanium metal after soaking in SBF for 5 d (dotted circle: area of electron diffraction and EDX analysis).

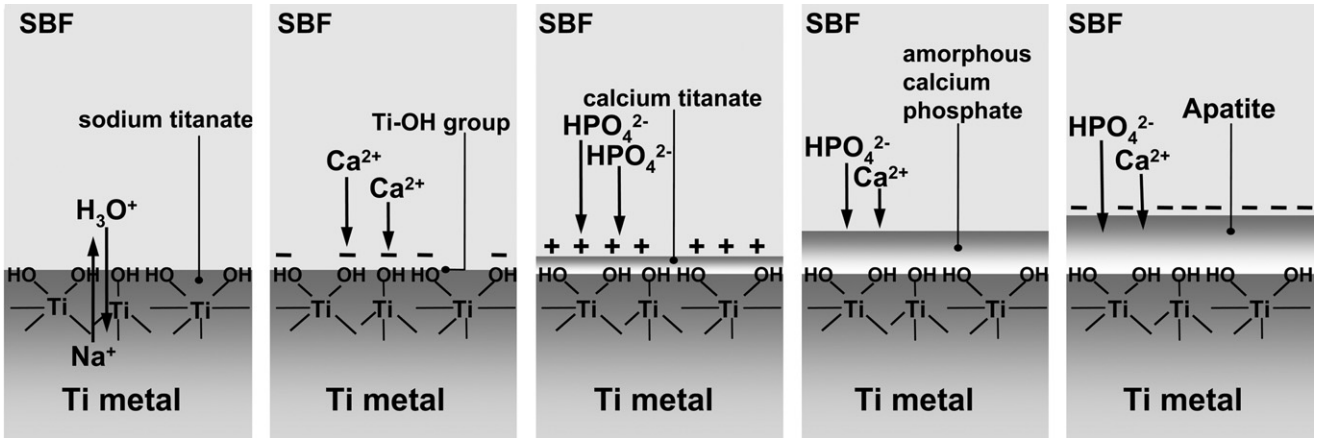

Fig. 6. Schematic representation of mechanism of apatite formation on NaOH- and heat-treated Ti metal in SBF.

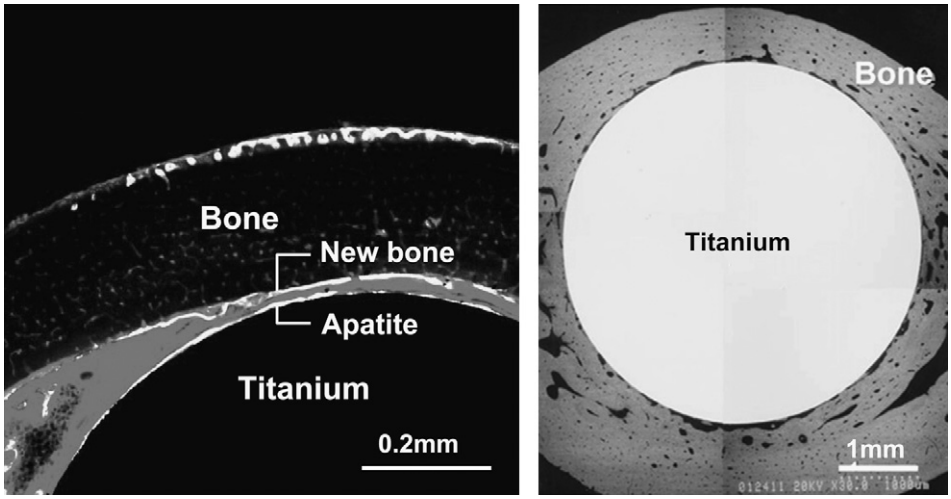

Fig. 7. Confocal laser scanning micrograph (left) and SEM phonograph (right) of cross-section of NaOH- and heat-treated Ti metal implanted into rabbit femur for 3 and 12 weeks, respectively.

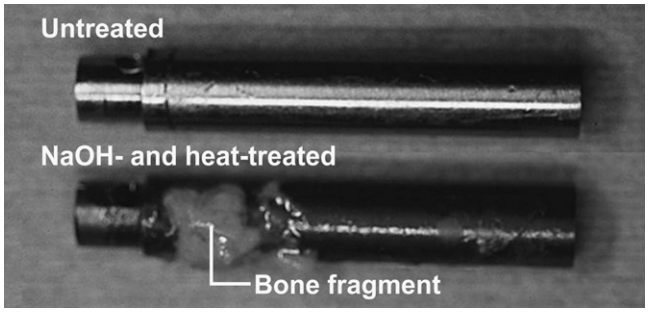

Fig. 8. Untreated and NaOH- and heat-treated titanium rods pulled out from intramedullar canal of rabbit femur at 12 weeks after implantation.

Detailed mechanism of this osteoinduction is also an interesting subject to be studied in future. However, it is already known that a phenomenon common to all hitherto reported osteoinductive materials is the formation of bonelike apatite on their surfaces in the living body. It is speculated that some biomolecules incorporated into the surface apatite layer during its formation might play an important role in inducing bone formation. This indicates that materials that form apatite on their surfaces in the living body can exhibit osteoconductivity as well as osteoinductivity when the pore structure is controlled. Then the osteoinduction also could be interpreted in terms of surface chemistry.

6. Development of novel bioactive composites

Various novel bioactive composites can also be derived from bioactive materials newly developed based on surface chemistry.

For example, fine fibres constituting a polyethylene terephthalate (PET) fabric can be modified with a thin titanium oxide layer on their surfaces by a sol–gel method. Thus modified PET fabric can show high apatite-forming ability in SBF, after subjected to HCl treatment, as shown in Fig. 11.[39,40] This PET fabric could form apatite on individual fibres in the living body and bond to living bone. This might be useful as a flexible bone substitute.

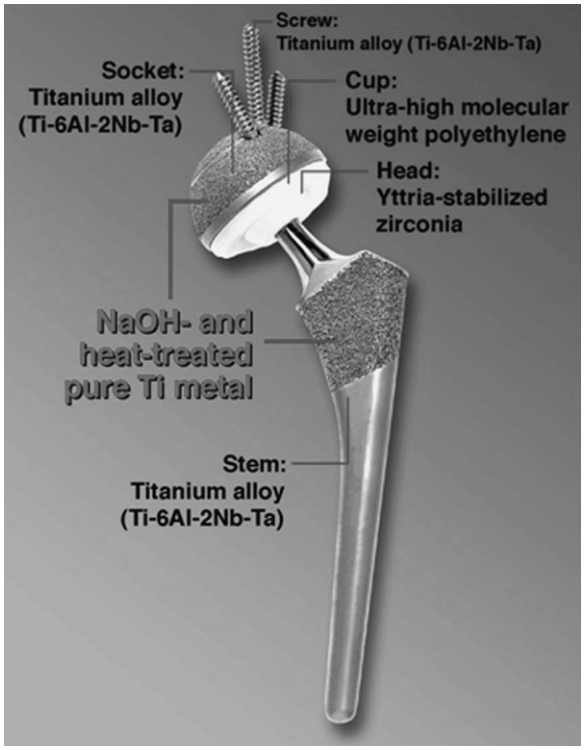

Fig. 9. Artificial total hip joint of titanium alloy formed with a bioactive porous titanium layer on its surface.

When a small amount of bioactive TiO_2 powder is added to the polymethyl methacrylate (PMMA) powder of PMMA cement and mixed with MMA monomer liquid, the solidified cement bonds to living bone tightly without the formation of surrounding fibrous tissue, as shown in Fig. 12.[41] This bioactive cement might be useful as a self-setting bone substitute.

7. Summary

Materials able to form an apatite on their surfaces in the living body bond to living bone through this apatite layer. The

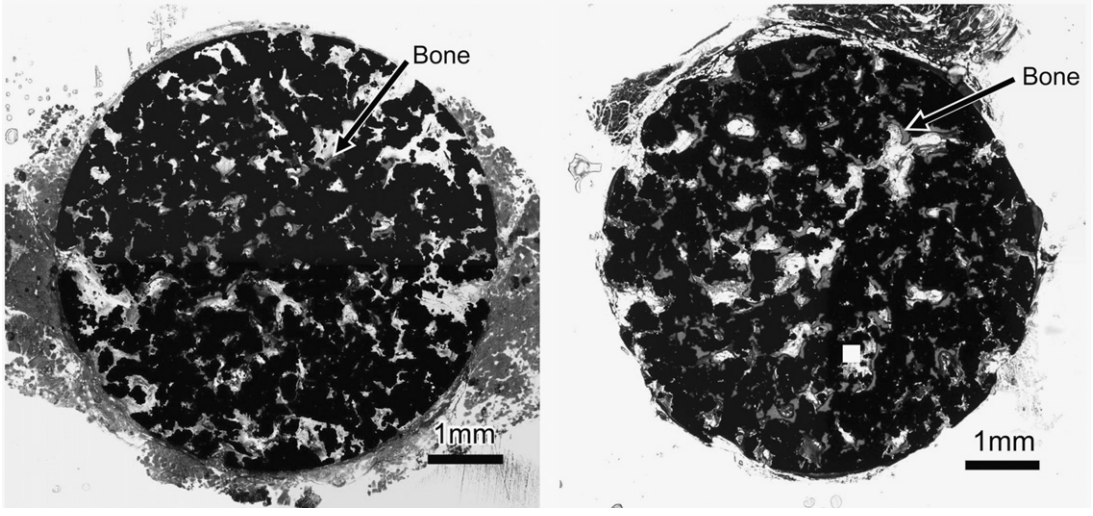

Fig. 10. Bone formation in porous Ti metal subjected to NaOH, HCl and heat treatments, 3 (left) and 12 (right) months after implantation into muscle of beagle dog.

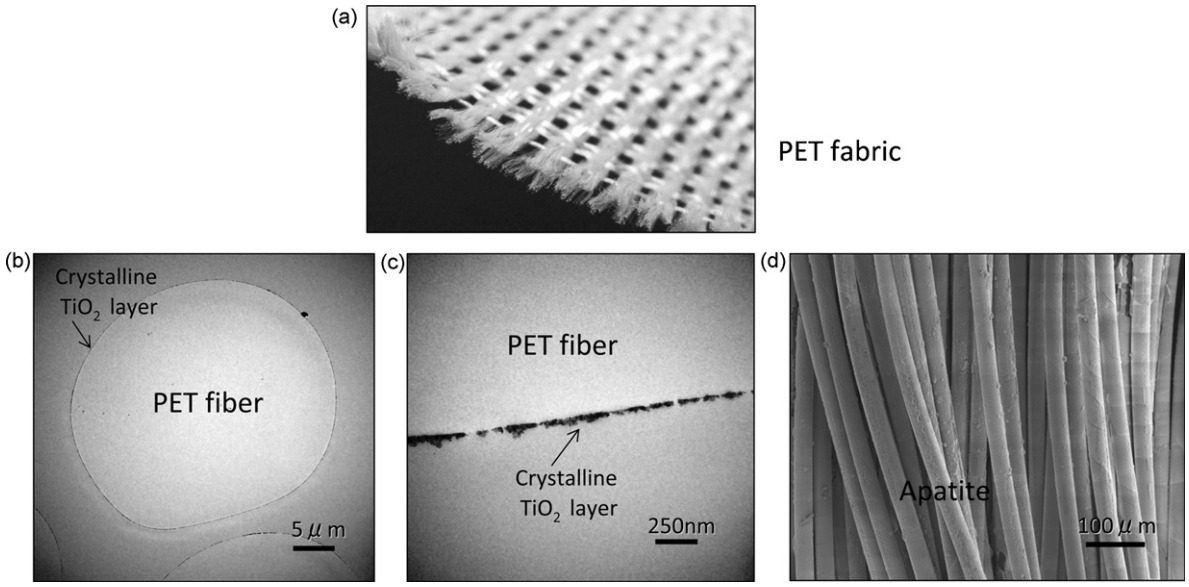

Fig. 11. PET fabric (a), crystalline TiO_2 layer (b and c) formed on PET fiber by a sol–gel method, and bonelike apatite (d) formed on PET fiber in SBF.

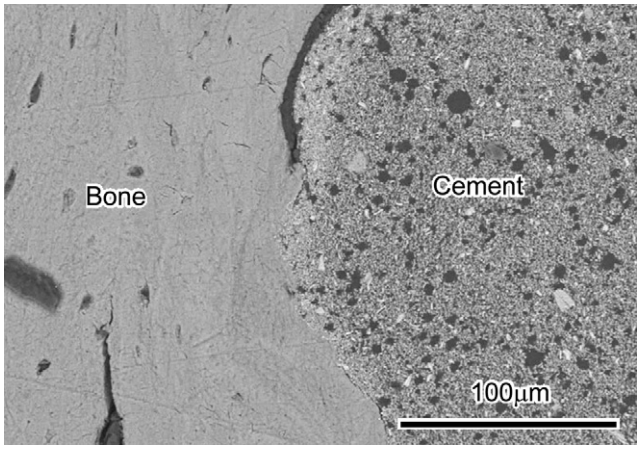

Fig. 12. Back-scattered electron image of interface between TiO_2-containing PMMA cement and bone, 6 weeks after implantation into rabbit tibia.

apatite-forming ability of a material can be evaluated in SBF without the need for animal experiments. Examination in SBF showed that various functional groups such as SiOH, TiOH, ZrOH, NbOH, TaOH, PO_4H_2, COOH and SO_3H are effective for apatite nucleation in the body environment. These findings indicate that various bioactive materials can be developed from ceramics, metals and organic polymers by modifying their surfaces with functional groups effective for apatite nucleation. Bioactive titanium metal and its alloys were developed by modifying their surfaces with sodium titanates and already clinically used as artificial hip joint.

A porous titanium metal modified with a titanium oxide on its pore wall also showed high apatite forming ability in SBF, resulting in high osteoconductivity as well as osteoinductivity. If detailed mechanisms of this high apatite forming ability as well as osteoinductivity are revealed in future, quite new bioactive material could be developed.

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