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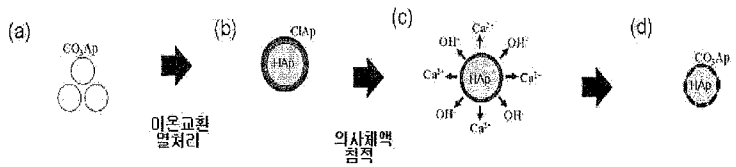
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(54) Title: BIOACTIVE APATITE HAVING DUAL STRUCTURE AND METHOD FOR PREPARING THE SAME

FIG. 7



(57) Abstract: Disclosed herein are a dual-structured bioactive apatite and a preparation method thereof. More specifically, disclosed are: a dual-structured bioactive chloro- /hydroxy-apatite, which has an inner portions formed of hydroxy apatite, and chlorinated apatite formed on the surface of hydroxyapatite; a dual-structured bioactive low crystalline carbonated hydroxyapatite, which has an inner portions formed of hydroxyapatite, and the low crystalline carbonated apatite formed on the surface of hydroxyapatite; methods for preparing said bioactive apatites; and bone graft substitutes containing any one of said dual-structured bioactive apatites.

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BIOACTIVE APATITE HAVING DUAL STRUCTURE AND METHOD FOR PREPARING THE SAME

TECHNICAL FIELD

The present invention relates to a dual-structured(core-rim) bioactive apatite and a method for preparing the same, and more particularly, to a dual-structured(core-rim) bioactive chloro-/hydroxy-apatite, a dual-structured bioactive low crystalline carbonated hydroxyapatite, and methods for preparing said dual-structured bioactive apatites.

BACKGROUND ART

A bone graft substitute (BGS) refers to a graft material that is used to substitute for bone tissue defects caused by various dental diseases or traumas, disease-related degeneration, tissue loss and the like, so as to fill pore spaces in the bone tissue and to promote the formation of new bone. The best graft is generally known to be autogenous bone graft, but the autogenous bone graft has problems in that it requires a secondary surgical operation, is difficult to obtain the required amount, is difficult to carry out at small-scale hospitals, and has a possibility that will make the pain and morbidity severe.

For this reason, various substitutes, including donated human bones, heterogenous bones, and artificially synthesized materials made of a bone component, hydroxyapatite, have been used for grafting. Commercially available bone substitutes are advantageous in that they are available in various forms, including powder, gel, slurry/putty, tablets, chips, morsels, pellets, sticks, sheets and blocks, are homogeneous, have a low risk with respect to infection and disease, eliminate the risk of pains resulting from the collection of a patient's own bones for grafting,

and have reduced limitations in size. However, these commercial bone substitutes have various problems. For example, because their structure is significantly different from the physical structure of human bone, they have a slow tissue regeneration rate.

In an attempt to solve these problems, bone minerals obtained by physicochemically treating animal bones having a structure similar to that of human bones so as to remove organic substances, have been processed such that they could be used in dental or orthopedic surgical operations. A typical example thereof may include Bio-Oss® commercially available from Biomaterials Geistlich.

A method for preparing said bone graft substitute using animal bones comprises the steps of: treating the thighbone of a bovine animal in a solvent having a boiling point of 80~120℃ to remove lipids; adding ammonia or primary amine to the treated bone to remove proteins and organic substances, thus obtaining bone mineral; and heating the bovine mineral at a high temperature of 250~600℃ for a few hours, followed by drying (US 5,167,961 and 5,417,975).

In another prior art, a method for preparing hydroxyapatite granules for biomaterials is known (Korean Patent 10-0498759). In this registered Korean patent, spherical hydroxyapatite powder can be effectively prepared in an aqueous solution and can be used as bone defect fillers, drug carriers and implant surface-treating agents. However, the hydroxyapatite powder has problems in that it does not show bioactivity and has low biocompatibility.

Meanwhile, the applicant previously developed a method for preparing a bone graft substitute, the method comprising treating bovine bone with sodium hypochlorite and treating the treated bone at low temperature, thus obtaining a low crystalline carbonated apatite phase from the bone without causing a great change in the physical/chemical properties thereof (Korean Patent 0635385). The method

disclosed in said patent comprises chemically inactivating prion protein causing bovine spongiform encephalopathy. However, in view of the fact that prion protein is completely inactivated, only when it is thermally treated at a temperature higher than 900 ℃, the bone graft substitute prepared according to said patent may have the risk of bovine spongiform encephalopathy.

Accordingly, the present inventors have made many efforts to solve the above-described problems occurring in the prior art and, as a result, have prepared a dual-structured bioactive chloro-/hydroxy-apatite by ion exchanging animal bone, having a structure similar to that of human bone, in a chloride ion-containing aqueous solution, and thermally treating the ion-exchanged bone at high temperature, and found that, when the dual-structured bioactive apatite is implanted *in vivo*, the chlorinated apatite layer on the surface thereof is dissolved, while a low crystalline carbonated apatite is formed on the surface of the hydroxyapatite under the chlorinated apatite layer, so as to increase the osteoconductivity of the bioactive apatite. Also, the present inventors have found that, when the dual-structured bioactive chloro-/hydroxy-apatite is soaked *in vitro* in a solution supersaturated with respect to apatite, low crystalline carbonated apatite is formed on the surface of high crystalline hydroxyapatite so as to increase the osteoconductivity of the bioactive apatite, thereby completing the present invention.

SUMMARY OF INVENTION

It is a main object of the present invention to provide a dual-structured(core-rim) bioactive chloro-/hydroxy-apatite, in which, when the bioactive apatite is implanted *in vivo*, the hydroxyapatite is dissolved, while low crystalline carbonated apatite is produced on the surface of the hydroxyapatite, as well as a method for preparing the same.

Another object of the present invention is to provide a dual-structured bioactive

low crystalline carbonated hydroxyapatite, in which a low crystalline carbonated apatite is formed on the surface of the hydroxyapatite by soaking the dual-structured bioactive chloro-/hydroxy-apatite, in a solution supersaturated with respect to apatite, and thus peptides, proteins, fibrins, bone morphogenetic factors, bone growth agents and the like can readily adhere to the bioactive apatite, as well as a method for preparing the same.

To achieve the above objects, in one aspect, the present invention provides a method for preparing a dual-structured bioactive chloro-/hydroxy-apatite, the method comprising the steps of: (a) boiling animal bone, from which blood components have been removed, in deionized water, to remove fats and proteins from the bone, and drying the boiled bone; (b) grinding the dried bone, immersing and shaking the ground bone in a volatile polar organic solvent, removing the organic solvent from the bone, and drying the bone; (c) treating the dried bone with a chloride ion-containing aqueous solution to remove proteins from the bone and, at the same time, exchange carbonate ions, present in a certain thickness of carbonated apatite layer on the surface of bone, with chloride ions; and (d) thermally treating the ion-exchanged bone at 900~1200℃ to remove lipids and proteins from the bone, thus obtaining a dual-structured bioactive chloro-/hydroxy-apatite, in which the chlorinated apatite formed on the surface of the hydroxyapatite.

In another aspect, the present invention provides a dual-structured bioactive chloro-/hydroxy-apatite, which is prepared according to said method, which has an inner portion formed of the hydroxyapatite, and the chlorinated apatite formed on the surface of the hydroxyapatite.

In still another aspect, the present invention provides a method for preparing a dual-structured bioactive low crystalline carbonated hydroxyapatite, the method comprising the steps of: soaking said dual-structured bioactive chloro-/hydroxy-apatite in a solution supersaturated with respect to apatite, so as to dissolve the

chlorinated apatite layer on the surface of the dual-structured bioactive chloro-/hydroxy-apatite, thus forming a low crystalline carbonated apatite on the surface of the hydroxyapatite.

In still another aspect, the present invention provides a dual-structured bioactive low crystalline carbonated hydroxyapatite, prepared by said method, which has an inner portion formed of the hydroxyapatite, and the low crystalline carbonated apatite formed on the surface of the hydroxyapatite.

In the present invention, said organic solvent is preferably a mixed solvent of chloroform/methanol, and the chloride ion-containing aqueous solution is preferably an aqueous sodium hypochlorite solution or an aqueous sodium chloride solution, which has a chloride ion concentration of 30-80 wt%.

In still another aspect, the present invention provides a bone graft substitute, which contains the dual-structured bioactive chloro-/hydroxy-apatite, and a bone graft substitute, which contains the dual-structured bioactive low crystalline carbonated hydroxyapatite.

In the present invention, the bone graft substitute is preferably formulated by adding, to the dual-structured bioactive apatite, at least one biologically active substance selected from the group consisting of bone growth-promoting factors, peptides and proteins inducing stimulation of bone tissue fromation, fibrins, bone morphogenetic factors, bone growth agents, chemotherapeutic agents, antibiotics, analgesics, bisphosphonates, strontium salts, fluorine salts, magnesium salts and sodium salts.

In the present invention, the bone graft substitute is preferably formulated by adding, to the dual-structured bioactive apatite, at least one compound selected from the group consisting of hyaluronic acid, chondroitin sulfate, alginic acid,

chitosan, collagen, hydroxyapatite, calcium carbonate, calcium phosphate, calcium sulfate, and ceramics.

Other features and aspects of the present invention will be apparent from the following detailed description and the appended claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows scanning electron microscope images of the inventive dual-structured bioactive chloro-/hydroxy-apatite powder (a: 100x photograph; and b: 1,000x photograph).

FIG. 2 shows the results of XRD measurement of the inventive dual-structured bioactive chloro-/hydroxy-apatite powder.

FIG. 3 shows scanning electron microscope images (10,000x), taken after the inventive dual-structured bioactive chloro-/hydroxy-apatite powder was soaked in simulated body fluid at varying time points (a: 0 hr, b: 3 hr, c: 6 hr, and d: 7 days).

FIG. 4 shows the results of XRD measurement, obtained before and after the inventive dual-structured bioactive chloro-/hydroxy-apatite powder was soaked in simulated body fluid for 7 days.

FIG. 5 shows the results of FT-IR measurement, obtained before and after the inventive dual-structured bioactive chloro-/hydroxy-apatite powder was soaked in simulated body fluid for 7 days.

FIG. 6 shows the changes in calcium concentration, phosphorus concentration and pH, and the value of ionic activity products calculated based on said concentrations and pH, when the inventive dual-structured bioactive chloro-/hydroxy-apatite

powder was soaked in simulated body fluid (a: Ca, b: P, c: pH, and d: IAP).

FIG. 7 schematically shows the inventive dual-structured bioactive chloro-/hydroxy-apatite and a mechanism in which a low crystalline carbonated apatite is produced on the surface of the inventive dual-structured bioactive chloro-/hydroxy-apatite powder, when the bioactive apatite is soaked in simulated body fluid.

FIG. 8 shows a scanning electron microscope photograph of an apatite, obtained by treating xenogeneic bone with aqueous sodium chloride solution, and then thermally treating the bone at 1000℃ for 3 hours (1,000x photograph).

FIG. 9 shows a scanning electron microscope photograph, taken after an apatite, prepared by treating xenogeneic bone with aqueous sodium chloride solution, and then thermally treating the bone at 1000℃ for 3 hours, was soaked in simulated body fluid for 7 days (1,000x photograph).

FIG. 10 shows a scanning electron microscope photograph of powder obtained by treating xenogeneic bone with sodium hypochlorite, washing the treated bone with distilled water, and then thermally treating the washed bone at 1000℃ for 3 hours (10,000x photograph).

FIG. 11 shows a scanning electron microscope photograph taken after powder, prepared by treating xenogeneic bone with sodium hypochlorite, washing the treated bone with distilled water, and then thermally treating the washed bone at 1000℃ for 3 hours, was soaked in simulated body fluid for 7 days (10,000x photograph).

FIG. 12 shows the results of XRD measurement, obtained before and after the inventive dual-structured bioactive chloro-/hydroxy-apatite powder was washed with distilled water for 1 hour.

FIG. 13 shows a scanning electron microscope photograph taken after the inventive dual-structured bioactive apatite chloro-/hydroxy-apatite was soaked in distilled water for 1 hour, completely dried and then soaked again in simulated body fluid for 7 days (1,000x photograph).

FIG. 14 shows a scanning electron microscope photograph of xenogeneic bone, which was soaked in an aqueous sodium hydroxide solution, and then thermally treated at 1000℃ for 3 hours (100x photograph).

FIG. 15 shows a scanning electron microscope photograph of xenogeneic bone, which was soaked in an aqueous sodium hydroxide solution, thermally treated at 1000℃ for 3 hours, and then soaked in simulated body fluid for 7 days (10,000x photograph).

DETAILED DESCRIPTION OF THE INVENTION, AND PREFERRED EMBODIMENTS

In one aspect, the present invention relates to a dual-structured(core-rim) bioactive chloro-/hydroxy-apatite, which has an inner portion formed of the hydroxyapatite, and the chlorinated apatite formed on the surface of the hydroxyapatite; and a method for preparing the dual-structured bioactive chloro-/hydroxy-apatite.

In order to prepare the dual-structured bioactive chloro-/hydroxy-apatite, animal bone, from which blood components have been removed, is first boiled in deionized water to remove fats and proteins and dried. The dried bone is immersed and shaken in a volatile polar organic solvent, the organic solvent is removed, and then the bone is dried. The organic solvent is preferably a mixed solvent of chloroform/methanol, but the scope of the present invention is not limited thereto.

The bone is soaked in a chloride ion-containing aqueous solution for 1 hour to remove proteins remaining therein, and the carbonate ions of carbonated apatite present in bone are exchanged with chloride ions. The chloride ion-containing aqueous solution can be prepared by dissolving all chloride ion-containing inorganic salts in water, and an aqueous sodium hypochlorite solution or an aqueous sodium chloride solution is preferably used as the chloride ion-containing aqueous solution. The chloride ion-containing aqueous solution preferably has a chloride ion concentration of 30-80 wt%. If the chloride ion concentration of the chloride ion-containing aqueous solution is less than 30 wt%, the efficiency for removing proteins will be reduced, and if it is more than 80 wt%, the yield of the bioactive apatite will be reduced.

After the above-described procedure is repeated several times, the bone is subjected to ion exchange for 12 hours to replace the carbonate ions present in a certain thickness of carbonated apatite on the bone surface, with chloride ions, and dried at room temperature. Herein, the layer, in which the carbonate ions are exchanged with the chloride ions, preferably has a thickness of less than 100 ㎛. The ion-exchanged bone is thermally treated at 900~1200℃ for 1-6 hours to completely remove lipids and proteins, thus obtaining a dual-structured bioactive chloro-/hydroxy-apatite, which has a given thickness of the chlorinated apatite formed on the surface of the hydroxyapatite. The form of dual-structured bioactive chloro-/hydroxy-apatite is preferably selected from the group consisting of powders, tablets, chips, morsels, pellets, sticks, sheets, blocks and scaffolds.

In another aspect, the present invention relates to a dual-structured bioactive low crystalline carbonated hydroxyapatite, which has an inner portion formed of the hydroxyapatite, and a low crystalline carbonated apatite formed on the surface of the hydroxyapatite; and a method for preparing the dual-structured bioactive low crystalline carbonated hydroxyapatite.

In order to prepare a dual-structured bioactive low crystalline carbonated hydroxyapatite according to the present invention, the above-prepared dual-structured bioactive chloro-/hydroxy-apatite is soaked in a solution supersaturated with respect to apatite. As a result, the chlorinated apatite layer on the surface of the dual structured chlorinated hydroapatite is dissolved, while calcium and hydroxide ions are released from bone, and thus the ionic activity product of apatite in the aqueous solution is increased, so that a low crystalline carbonated crystal is formed on the surface of the hydroxyapatite, thus obtaining a dual-structured bioactive low crystalline carbonated hydroxyapatite apatite.

In the present invention, the solution supersaturated with respect to apatite preferably has a solubility product of $5.5 \times 10^{-118} \sim 10^{-90}$ for apatite.

In still another aspect, the present invention relates to a bone graft substitute, which contains the dual-structured bioactive chloro-/hydroxy-apatite, and a bone graft substitute, which contains the dual-structured bioactive low crystalline carbonated hydroxyapatite.

As used herein, the term "bone graft substitute" refers to a bone graft substitute composition, which is a material for filling spaces in bone tissue. The bone graft substitute can be used in the form of putty, paste, formable strips, blocks, chips, etc., which are formed by compressing, compacting, pressably contacting, packing, squeezing or tamping the bone powder into the desired shape. Also, it can be used in the form of gel, granules, paste, tablets, pellets, etc., which are formed using chemical additives, and it can be used in a powder form as it is.

If the bone graft substitute is used in the above-described forms, it is preferable to add biologically active substances thereto. Examples of the biologically active substances, which can be used in the present invention, include a bone growth-

promoting factor, peptides and proteins inducing promotion of bon tissue generation, a fibrin, a bone morphogenic factor, a bone growth agent, a chemotherapeutic agent, an antibiotic, an analgesic, a bisphosphonate, a strontium salt, a fluorine salt, a magnesium salt, and a sodium salt.

Examples of the growth factor, which can be used in the present invention, include BMP (bone morphogenic protein), PDGF (platelet-derived growth factor), TGF-beta (transgenic growth factor), IGF-I (insulin-like growth factor), IGF-II, FGF (fibroblast growth factor) and BGDF-II (beta-2-microglobulin). Examples of peptides and proteins inducing promotion of bon tissue generation, which can be used in the present invention, include various peptides containing an RGD sequence, and various proteins such as collagen and fibrinogen. Examples of the bone morphogenic factor, which can be used in the present invention, include osteocalcin, bonesialo protein, osteogenin, BMP and the like. The bone growth agent can be used without any particular limitation as long as it is harmless to the human body and promotes bone growth. Examples of the bone growth agent, which can be used in the present invention, include nucleic acids that promote bone formation, and antagonists of substances that inhibit bone formation.

Examples of chemical additives, which are used to form the bone graft substitute in the present invention, include hyaluronic acid, chondroitin sulfate, alginic acid, chitosan, collagen, hydroxyapatite, calcium carbonate, calcium phosphate, calcium sulfate, and ceramics. Depending on the kind of the additives, the bone graft substitute can be formed in the shape of gel, strips, granules, chips, pellets, tablets, paste, etc.

Examples

Hereinafter, the present invention will be described in further detail with reference to examples. It is to be understood, however, that these examples are for

illustrative purposes only and are not to be construed to limit the scope of the present invention.

Example 1: Preparation of dual-structured bioactive apatite using sodium hypochlorite

[Pretreatment and grinding step]

A bovine femoral bone was cut to a size of 5 cm^3 using a bone cutter. The cut bone pieces were immersed in deionized water for 24 hours to remove blood components present in the bone. The bone pieces washed with deionized water were boiled for 72 hours while replacing the deionized water at 12-hr intervals, thus primarily removing lipids and proteins present in the bones. The bone pieces, from which the lipids and proteins have primarily been removed were completely dried in an oven at 60℃ for 24 hours, and then ground to a size of less than 1.5 mm using a bone mill.

[Defatting step]

To 1 g of the ground bone powder, 20 ml of a mixed solvent of chloroform and methanol (1:1 v/v) was added and the solution was shaken at a rotating speed of 120 rpm for 24 hours so as to defat the bone powder. In order to remove the solvent remaining in the defatted bone powder, deionized water was added to the bone powder in a weight ratio of 50:1, and then the solution was shaken at 120 rpm for 12 hours, thus removing the solvent remaining in the powder. At this time, the deionized water was replaced with fresh deionized water at 2-hr intervals in order to increase washing efficiency. The washed bone powder was completely dried in an oven at 60℃.

[Ion exchange step]

40 g of defatted bone powder was added to 100 g of aqueous sodium hypochlorite solution, and the mixture was shaken at a revolution speed of 120 rpm for 1 hour to

remove proteins from the bone powder and, at the same time, exchange the carbonate ions of carbonated apatite present in bone, with chloride ions. Then, the same procedure as described above was performed once more, and then the bone was subjected to ion exchange for 12 hours in the same conditions as described above, so as to completely replace carbonate ions, present in a certain thickness of the carbonated apatite layer on the surface of the bone powder, with chloride ions, and was completely dried at room temperature.

[Thermal treatment step]

The ion-exchanged bone powder was thermally treated at 1000℃ for 3 hours using an electric furnace at a heating rate of 2℃/min and cooled down in the furnace to completely remove lipids and proteins from the bone powder, thus obtaining a dual-structured chloro-/hydroxy-apatite powder, which has the chlorinated apatite layer formed on the surface of the hydroxyapatite.

[Sieving step]

The thermally treated powder particles were sieved through a sieve having a pore size of 212-1000㎛ so as to select powder particles having a size of 212-1000㎛.

[Step of coating low crystalline carbonated apatite]

The sieved dual-structured chloro-/hydroxy-apatite powder was soaked in simulated body fluid (Kokubo, T. *et al.*, *J. Biomed. Mater. Res.*, 24:721-34, 1990), that is, a solution having an ionic activity product (IAP) of 10^{-95} for apatite. As a result, the chlorinated apatite layer was dissolved, while calcium ions and hydroxide ions were released from the bone, and the ionic solubility product of apatite in the solution was increased, so that a low crystalline carbonated layer was formed on the surface of the hydroxyapatite, thus obtaining a dual-structured low crystalline carbonated hydroxyapatite.

The dual-structured bioactive chloro-/hydroxy-apatite powder, prepared through

the ion exchange step, was analyzed with a scanning electron microscope. As a result, it was observed that the abnormal growth of the apatite particles occurred to develop hexagonal pillar-like particles having a size of more than 50 ㎛ (FIG. 1).

Also, the surface layer of dual-structured bioactive chloro-/hydroxy-apatite powder, prepared through the ion exchange step, was analyzed using XRD. As a result, it could be observed that the surface of the powder was mostly formed of the chlorinated apatite phase, and a portion of the surface was formed of the hydroxyapatite phase (FIG. 2).

The dual-structured bioactive chloro-/hydroxy-apatite powder was soaked in simulated body fluid and analyzed with a scanning electron microscope at varying time points. As a result, as shown in FIG. 3, the dual-structured bioactive apatite particles had a smooth surface before soaked (FIG. 3a), and the surface thereof was dissolved with the passage of soaking time to form the concavo-convex shape (FIG. 3b). After the soaking time has further elapsed, lobule-like crystals started to be produced on the surface thereof (FIG. 3c), and at the 7^{th} day after soaking, the surfaces of all the particles were covered with nanometer-sized apatite particles (FIG. 3d).

The dual-structured bioactive apatite chloro-/hydroxy-apatite was analyzed using XRD, before and after it was soaked in simulated body fluid. As a result, it could be observed that the chlorinated apatite phase on the surface before soaked was converted to the hydroxyapatite phase after it is soaked, and fine particles produced on the powder surface was hydroxyapatite particles (FIG. 4).

Also, the dual-structured bioactive apatite chloro-/hydroxy-apatite was analyzed by FT-IR, before and after it was soaked in simulated body fluid. As a result, before soaked, no carbonate group was detected, suggesting that the chlorinated apatite was not converted, but after soaked, a carbonate group was detected, suggesting

that carbonated apatite was produced (FIG. 5).

Putting the XRD results of FIG. 4 and the FT-IR results of FIG. 5 together, it could be confirmed that fine particles formed in the simulated body fluid were low crystalline carbonated apatite particles having physical and chemical properties similar to those of human bone.

When the dual-structured bioactive chloro-/hydroxy-apatite powder was soaked in the simulated body fluid, the concentrations of calcium and phosphorus in the simulated body fluid were analyzed by ICP-AES at varying time points, and the pH value of the simulated body fluid was measured at varying time points. Based on such data, the ionic activity produce of apatite in the simulated body fluid was calculated. As a result, as shown in FIG. 6, it could be confirmed that, when the dual-structured bioactive chloro-/hydroxy-apatite was soaked in the simulated body fluid, the chlorinated apatite layer on the surface thereof was dissolved, and calcium and hydroxide ions were released from the bioactive apatite, but phosphorus was not dissolved. Also, it could be seen that the ionic activity product of apatite in the solution was increased due to the release of calcium and hydroxide ions, so that a low crystalline carbonated apatite was formed on the surface.

FIG. 7 schematically shows the inventive dual-structured bioactive chloro-/hydroxy-apatite and a low crystalline carbonated apatite formed on the surface of the dual-structured bioactive chloro-/hydroxy-apatite, when the bioactive apatite is soaked in simulated body fluid. As shown in FIG. 7, when carbonated apatite (CO_3Ap) ((a) of FIG. 7) present in defatted animal bone is ion-exchanged by treatment with sodium hypochlorite and is thermally treated at high temperature, chlorinated apatite (ClAp) is formed on the surface of hydroxyapatite (HAp) in the bone as shown in (b) of FIG. 7. When the ion-exchanged bone is soaked in a solution supersaturated with respect to apatite, the chlorinated apatite layer on the

bone surface is dissolved, while calcium and hydroxide ions are released from the bone as shown in (c) of FIG. 7, and calcium, phosphorus and carbonate ions, which are present in the aqueous solution, are combined with each other, so that a new low crystalline carbonated apatite (CO_3Ap) is formed on the surface of hydroxyapatite (HAp) as shown in (d) of FIG. 7.

Example 2: Preparation of dual-structured bioactive apatite using sodium chloride

An experiment was carried out in the same manner as in Example 1 using an aqueous sodium chloride solution instead of the aqueous sodium hypochlorite solution. Specifically, 10 g of sodium chloride was completely dissolved in 100 g of water, and 20 g of bone powder, which was defatted and then washed with water, was added to the aqueous sodium chloride solution, and the mixture was subjected to ion exchange for 12 hours while being shaken at a revolution speed of 120 rpm. Then, the ion-exchanged bone was thermally treated at 1000℃ to stably fix chloride ions to the apatite structure.

The dual-structured bioactive chloro-/hydroxy-apatite, obtained by treating the bone with the aqueous sodium chloride solution and thermally treating the ion-exchanged bone at 1000℃, was analyzed with a scanning electron microscope. As a result, it could be seen that, as in the case where sodium hypochlorite was used as a chloride ion source, the apatite particles abnormally grew, and thus had a size of more than 50 ㎛ (FIG. 8).

After the dual-structured bioactive chloro-/hydroxy-apatite, obtained by treating the bone with the aqueous sodium chloride solution and then thermally treating the ion-exchanged bone at 1000℃, was soaked in simulated body fluid for 7 days, the bioactive apatite was analyzed with a scanning electron microscope. As a result, it could be observed that low crystalline carbonated apatite crystals were formed on the surface of the dual-structured chloro-/hydroxy-apatite (FIG. 9).

Comparative Example 1

In this Comparative Example, hydroxyapatite was prepared by treating bone powder with sodium hypochlorite, washing the treated bone with water, and then thermally treating the washed bone.

[Pretreatment and grinding step]

A bovine femoral bone was cut to a size of 5 cm^3 using a bone cutter. The cut bone pieces were immersed in deionized water for 24 hours to remove blood components present in the bone. The bone pieces washed with deionized water were boiled for 72 hours while replacing the deionized water at 12-hr intervals, thus primarily removing lipids and proteins present in the bones. The bone pieces, from which the lipids and proteins have primarily been removed, were completely dried in an oven at 60℃ for 24 hours, and then ground to a size of less than 1.5 mm using a bone mill.

[Defatting step]

To 1 g of the ground bone powder, 20 ml of a mixed solvent of chloroform and methanol (1:1 v/v) was added and the solution was shaken at a rotating speed of 120 rpm for 24 hours so as to defat the bone powder. In order to remove the solvent remaining in the defatted bone powder, deionized water was added to the bone powder in a weight ratio of 50:1, and then the solution was shaken at 120 rpm for 12 hours, thus removing the solvent remaining in the powder. At this time, the deionized water was replaced with fresh deionized water at 2-hr intervals in order to increase washing efficiency. The washed bone powder was completely dried in an oven at 60℃.

[Ion exchange step]

40 g of defatted bone powder was added to 100 g of aqueous sodium hypochlorite

solution, and the mixture was shaken at a revolution speed of 120 rpm for 1 hour to remove proteins from the bone powder and, at the same time, exchange the carbonate ions of carbonated apatite present in the bone, with chloride ions. Then, the same procedure as described above was performed once more, and then the bone was subjected to ion exchange for 12 hours in the same conditions as described above, so as to replace carbonate ions present in a certain thickness of the carbonated apatite on the surface of the bone powder, with chloride ions. In order to remove sodium hypochlorite remaining in the surface, deionized water was added to the bone powder in a weight ratio of 50:1, and then the solution was shaken at 120 rpm for 72 hours, thus removing the remaining sodium hypochlorite. At this time, the deionized water was replaced with fresh deionized water at 2-hr intervals during 12 hours, and then the deionized water was replaced with fresh deionized water at 12-hr intervals. The washed bone powder was completely dried in an oven at 60℃.

[Thermal treatment step]

The ion-exchanged bone powder was thermally treated at 1000℃ for 3 hours using an electric furnace at a heating rate of 2℃/min.

The hydroxyapatite, obtained in this Comparative Example, was analyzed with a scanning electron microscope. As a result, the hexagonal pillar-like particles shown in FIG. 1 were not found, and angular particles having a size of about 1 ㎛ were observed in the hydroxyapatite (FIG. 10). Also, after the hydroxyapatite granules, obtained in this Comparative Example, were soaked in simulated body fluid for 1 week, the hydroxyapatite was analyzed with a scanning electron microscope. As a result, it could be observed that no low crystalline carbonated apatite crystal was produced in the hydroxyapatite (FIG. 11).

Comparative Example 2

In this Comparative Example, an experiment was carried out in order to examine whether a low crystalline carbonated apatite would be produced, when the dual-structured chloro-/hydroxy-apatite, prepared in Example 1, was washed with deionized water to dissolve the chlorinated apatite layer, and then soaked in simulated body fluid.

Before and after the dual-structured chloro-/hydroxy-apatite was washed with desionized water for 1 hour, it was analyzed using XRD. As a result, after the dual-structured apatite was washed with deionized water for 1 hour, no chlorinated apatite phase was observed, and only a pure hydroxyapatite phase was observed. This suggests that the dual-structured chloro-/hydroxy-apatite, prepared according to the above-described method, has a dual structure of an inner portion formed of hydroxyapatite and a surface layer formed of chlorinated apatite (FIG. 12).

Also, after the dual-structured chloro-/hydroxy-apatite was soaked in distilled water for 1 hour, completely dried, and then soaked in simulated body fluid for 7 days, it was observed with a scanning electron microscope. As a result, no low crystalline carbonated apatite was produced on the surface of the dual-structured apatite. This suggests that production of low crystalline carbonated apatite is attributable to dissolution of the chlorinated apatite layer (FIG. 13).

Comparative Example 3

In this Comparative Example, an experiment was carried out in order to demonstrate that, when bone powder is treated with a salt solution, which contains sodium ions, but contains no chloride ions, according to the method described in Example 1, a low crystalline carbonated apatite is not produced on the bone powder, even if the treated bone powder is soaked in a solution supersaturated with respect to apatite.

10 g of sodium hydroxide (NaOH) was dissolved in 100 g of water, and 20 g of bone powder was added thereto. Then, the mixture was treated according to the method of Example 1 and thermally treated at 1000℃ for 3 hours. As a result, the abnormal growth of fine apatite particles did not occur (FIG. 14). Even after the treated bone powder was soaked in simulated body fluid for 7 days, no low crystalline carbonated apatite was produced in fine apatite particles (FIG. 15). That is, as shown in FIGS. 14 and 15, it could be confirmed in this Comparative Example that no chlorinated apatite was produced, and in addition, a low crystalline carbonated apatite was not produced in a solution supersaturated with respect to apatite.

Test Example 1: Evaluation of specific surface area and peptide adsorption efficiency

The inventive dual-structured low crystalline carbonated hydroxyapatite powder, pure chlorinated apatite, and a xenogeneic bone consisting of a low crystalline carbonated apatite (Korean Patent 10-0635385), were measured for their specific surface areas using a BET tester. Also, these bone samples were measured for the adsorption of an RGD peptide using a flurocytometer.

The quantification of the RGB peptide was performed by labeling the RGD peptide with a fluorescent substance, non-specifically adsorbing the labeled RGD peptide onto the surface of apatite in PBS solution for 24 hours, separating the solution with a sonicator, collecting a given amount of a sample from the supernatant of the PBS solution, and quantifying the sample using a flurocytometer.

As a result, as shown in Table 1 below, it could be seen that the inventive dual-structured low crystalline carbonated hydroxyapatite powder had an increased specific surface area, thus resulting in an increased peptide adsorption efficiency, compared to those of the pure hydroxyapatite and the xenogeneic bone consisting

of a low crystalline carbonated apatite.

Table 1: Specific surface area and RGD peptide adsorption

	Specific surface area	RGD peptide adsorption
Example 1	large	large
Example 2	large	large
Comparative Example 1	small	small
Comparative Example 2	small	small
Comparative Example 3	small	small
Xenogeneic bone consisting of low crystalline carbonated apatite (Korean Patent 10-0635385)	medium	medium

As described above, when animal bone is ion-exchanged in a chloride ion-containing aqueous solution, such as an aqueous sodium hypochlorite solution or an aqueous sodium chloride solution, and then thermally treated at high temperature, the dual-structured chloro-/hydroxy-apatite, which has the chlorinated apatite layer formed on the surface of hydroxyapatite, can be obtained. When the obtained dual-structured apatite is soaked in a solution supersaturated with respect to apatite (e.g., simulated body fluid), the chlorinated apatite layer on the surface thereof is dissolved, calcium and hydroxide ions are released, and thus ionic activity product of the apatite in the solution is increased, thus obtaining a dual-structured low crystalline carbonated hydroxyapatite, which has low crystalline carbonated apatite formed on the surface of the hydroxyapatite.

However, it could be seen that, when bone powder was not thermally treated directly after being treated with sodium hypochlorite, but was treated with sodium hypochlorite, washed with water and then thermally treated, a low crystalline carbonated apatite was not produced in the simulated body fluid. Also, after the dual-structured carbonated hydroxyapatite was washed with distilled water, the chlorinated apatite phase on the surface thereof was completely dissolved, and thus was not observed and only pure hydroxyapatite phase was observed, and even

when the washed apatite was soaked in the simulated body fluid, a low crystalline carbonated apatite was not produced on the surface thereof. This suggests that the production of low crystalline carbonated apatite is attributable to dissolution of the chlorinated apatite layer.

INDUSTRIAL APPLICABILITY

As described in detail above, the present invention has an effect to provide a bioactive apatite, which allows peptides and proteins inducing regeneration of bone tissue to be readily adsorbed, and to have has increased osteoconductivity and improved biocompatibility, as well as a method for preparing said bioactive apatite. According to the present invention, a dual-structured bioactive chloro-/hydroxy-apatite, which is completely free of the risk of bovine spongiform encephalopathy, can be prepared by exchanging the carbonate ions of carbonated apatite, present in animal bone having a structure and components similar to those of human bone, with chloride ions in a chloride ion-containing aqueous solution, and then thermally treating the ion-exchanged bone at high temperature so as to effectively remove fats and organic substances harmful to the human body. In addition, even when the dual-structured bioactive chloro-/hydroxy-apatite is implanted *in vivo* without being coated with a low crystalline carbonated apatite, the chlorinated apatite layer is dissolved in blood, and the degree of supersaturation with respect to the apatite in blood is increased, so that the low crystalline carbonated apatite is formed on the surface of the hydroxyapatite, thus increasing the osteoconductivity of the bioactive apatite. Also, when the dual-structured bioactive chloro-/hydroxy-apatite is soaked in a solution supersaturated with respect to apatite, nano-sized low crystalline carbonated apatite particles having excellent osteoconductivity is coated on the bioactive apatite, so that stimulation of bone tissue regeneration can be induced, the adhesion efficiency of various peptides and proteins, which positively induce the regeneration of bone tissue, to the bioactive apatite, can be increased, and osteoconductivity of the bioactive apatite can be increased.

Although the present invention has been described in detail with reference to the specific features, it will be apparent to those skilled in the art that this description is only for a preferred embodiment and does not limit the scope of the present invention. Thus, the substantial scope of the present invention will be defined by the appended claims and equivalents thereof.

THE CLAIMS

What is Claimed is:

1. A method for preparing a dual-structured bioactive chloro-/hydroxy-apatite, the method comprising the steps of:

(a) boiling animal bone, from which blood components have been removed, in deionized water, to remove fats and proteins from the bone, and drying the boiled bone;

(b) grinding the dried bone, immersing and shaking the ground bone in a volatile polar organic solvent, removing the organic solvent from the bone, and drying the bone;

(c) treating the dried bone with a chloride ion-containing aqueous solution having a chloride ion concentration of 20~80 wt.%, to remove proteins from the bone and, at the same time, exchange carbonate ions, present in a certain thickness of carbonated apatite layer on the surface of bone, with chloride ions; and

(d) thermally treating the ion-exchanged bone at 900~1200℃ to remove lipids and proteins from the bone, thus obtaining a dual-structured bioactive chlorinated hydroyapatite, which has a chlorinated apatite formed on the surface of a hydroxyapatite.

2. The method for preparing a dual-structured bioactive chloro-/hydroxy-apatite according to claim 1, wherein said organic solvent is a mixed solvent of chloroform/methanol.

3. The method for preparing a dual-structured bioactive chloro-/hydroxy-apatite according to claim 1, the chloride ion-containing aqueous solution having a chloride ion concentration of 20~80 wt.% is an aqueous sodium hypochlorite solution or an aqueous sodium chloride solution.

4. A dual-structured bioactive chloro-/hydroxy-apatite, prepared by the method of any one claim among claims 1-3, which has an inner portions formed of hydroxyapatite, and chlorinated apatite formed on the surface of hydroxyapatite.

5. A bone graft substitute, which contains the dual-structured bioactive chloro-/hydroxy-apatite of claim 4.

6. The bone graft substitute according to claim 5, which is formulated by adding, to the dual-structured bioactive apatite, at least one biologically active substance selected from the group consisting of bone growth-promoting factors, peptides and proteins inducing stimulation of bone tissue regeneration, fibrins, bone morphogenetic factors, bone growth agents, chemotherapeutic agents, antibiotics, analgesics, bisphosphonates, strontium salts, fluorine salts, magnesium salts and sodium salts.

7. The bone graft substitute according to claim 5, which is formulated by adding, to the dual-structured bioactive apatite, at least one compound selected from the group consisting of hyaluronic acid, chondroitin sulfate, alginic acid, chitosan, collagen, hydroxyapatite, calcium carbonate, calcium phosphate, calcium sulfate, and ceramics.

8. A method for preparing a dual-structured bioactive low crystalline carbonated hydroxyapatite, the method comprising the steps of: soaking the dual-structured bioactive chloro-/hydroxy-apatite of claim 4 in a solution supersaturated with respect to apatite, so as to dissolve the chlorinated apatite layer present on the surface of the dual-structured bioactive chloro-/hydroxy-apatite, thus forming a low crystalline carbonated apatite on the surface of the hydroxyapatite.

9. The method for preparing a dual-structured bioactive low crystalline carbonated hydroxyapatite, wherein the solution supersaturated with respect to apatite has a

solubility product of 5.5 x 10^{-118}~10^{-90} for apatite.

10. A dual-structured bioactive low crystalline carbonated hydroxyapatite, prepared by the method of claim 8, which has an inner portions formed of hydroxyapatite, and a low crystalline carbonated apatite formed on the surface of hydroxyapatite.

11. A bone graft substitute, which contains the dual-structured bioactive low crystalline carbonated hydroxyapatite of claim 10.

12. The bone graft substitute according to claim 11, which is formulated by adding, to the dual-structured bioactive apatite, at least one biologically active substance selected from the group consisting of bone growth-promoting factors, peptides and proteins inducing stimulation of bone tissue regeneration, fibrins, bone morphogenetic factors, bone growth agents, chemotherapeutic agents, antibiotics, analgesics, bisphosphonates, strontium salts, fluorine salts, magnesium salts and sodium salts.

13. The bone graft substitute according to claim 11, which is formulated by adding, to the dual-structured bioactive apatite, at least one compound selected from the group consisting of hyaluronic acid, chondroitin sulfate, alginic acid, chitosan, collagen, hydroxyapatite, calcium carbonate, calcium phosphate, calcium sulfate, and ceramics.

DRAWINGS

FIG. 1

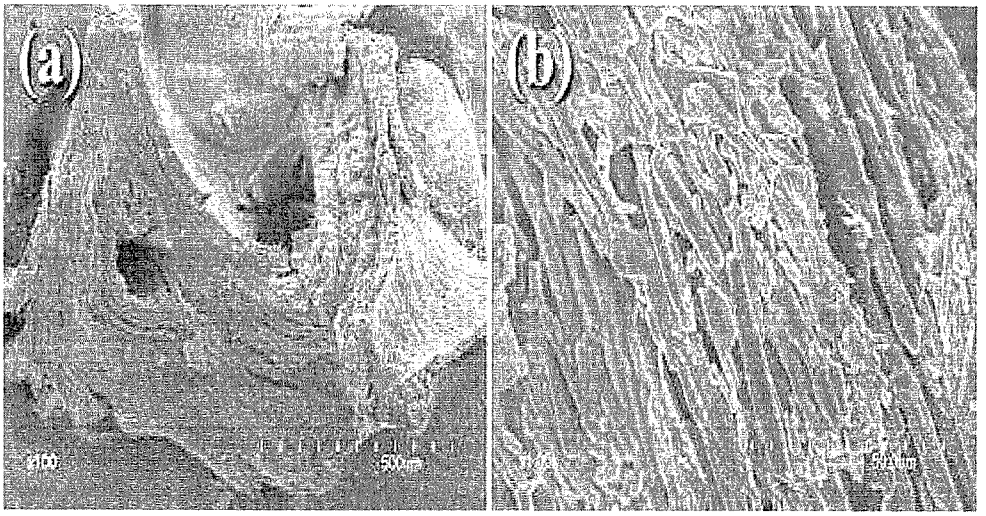


FIG. 2

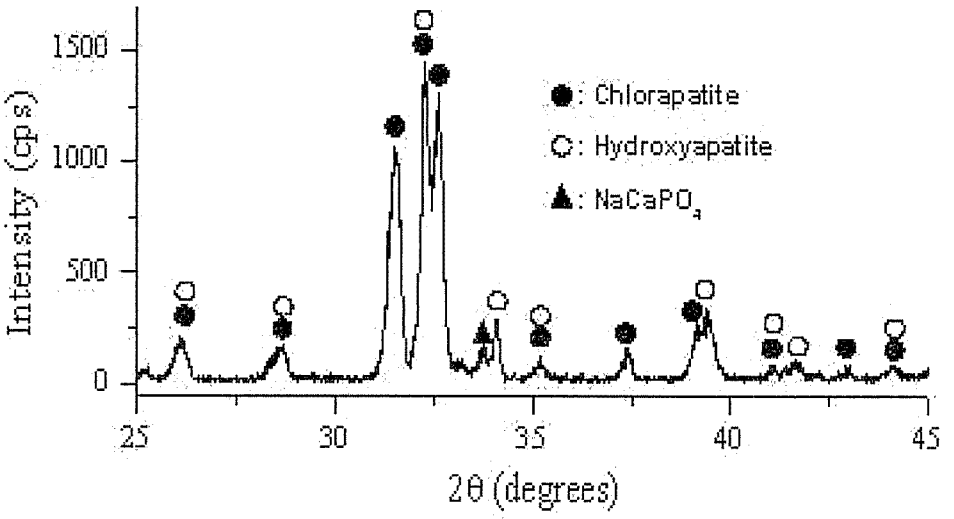


FIG. 3

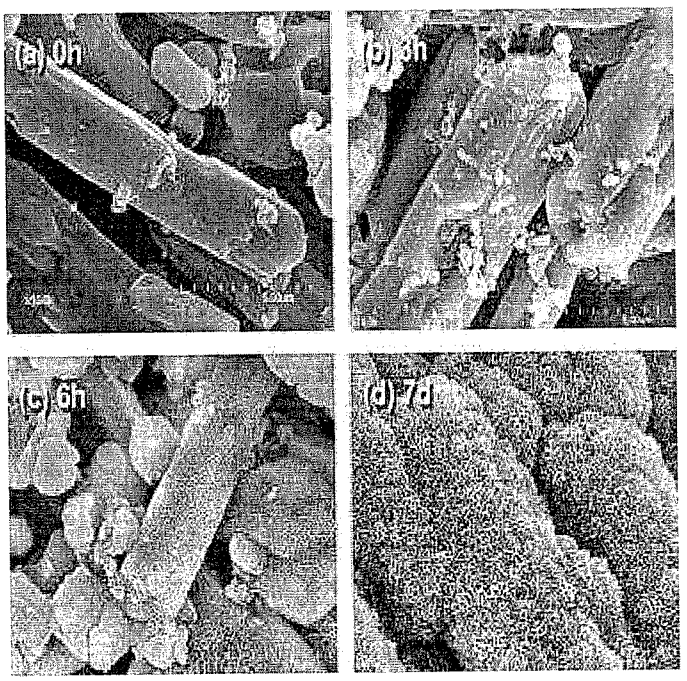

(a) 0h
(b) 3h
(c) 6h
(d) 7d

FIG. 4

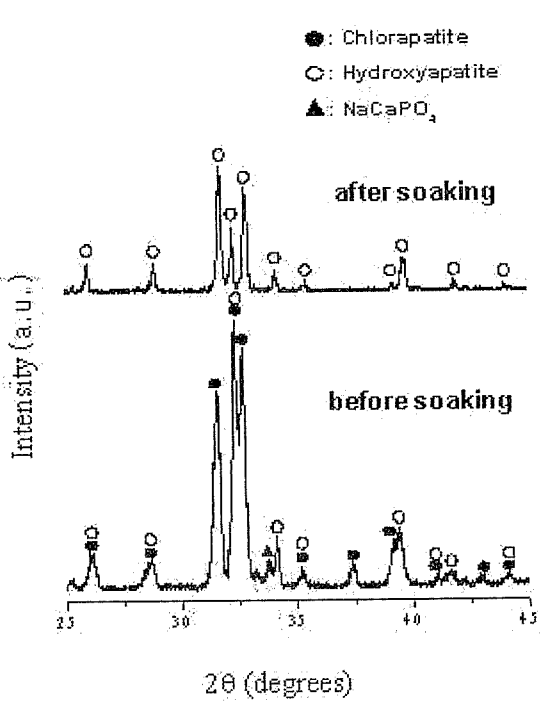

●: Chlorapatite
○: Hydroxyapatite
▲: NaCaPO4
after soaking
before soaking
Intensity (a.u.)
25
30
35
40
45
2θ (degrees)

FIG. 5

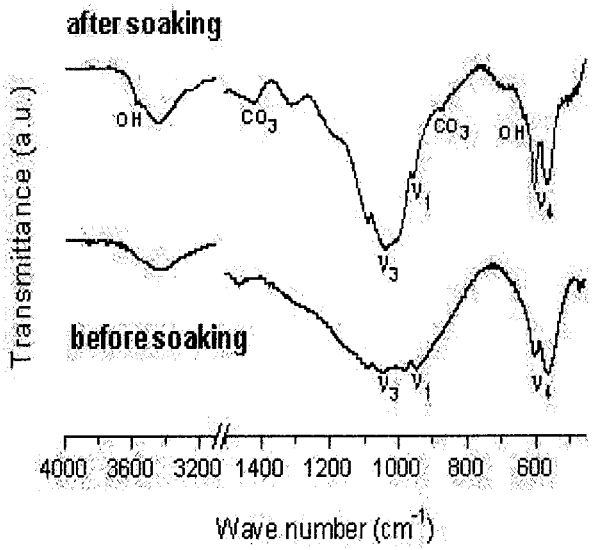

after soaking
before soaking
OH
CO3
CO3
OH
ν1
ν3
ν4
Transmittance (a.u.)
4000 3600 3200 1400 1200 1000 800 600
Wave number (cm-1)

FIG. 6

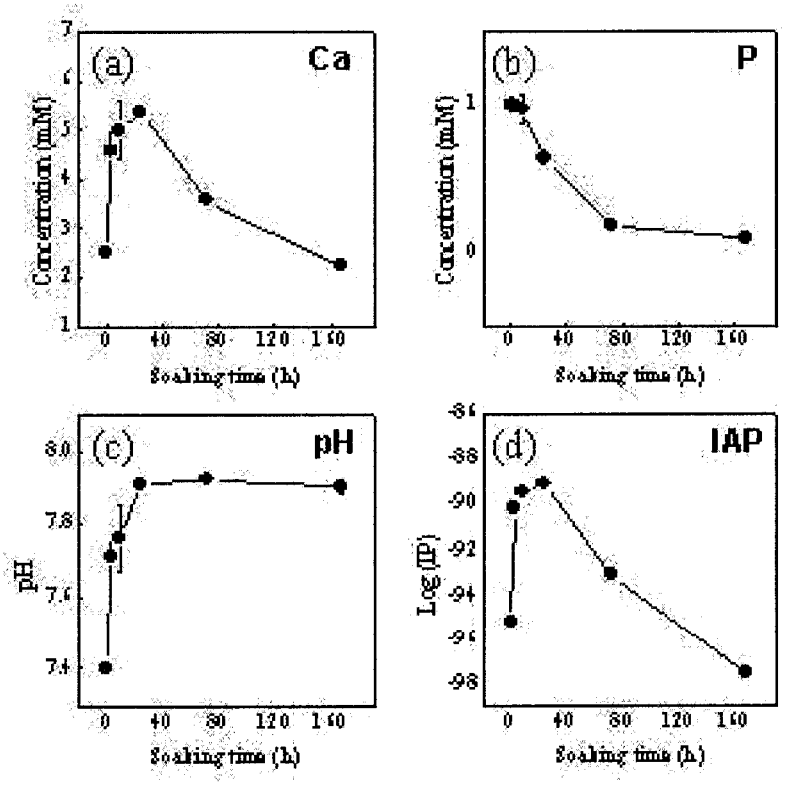

(a) Ca
Concentration (mM)
Soaking time (h)
(b) P
Concentration (mM)
Soaking time (h)
(c) pH
pH
Soaking time (h)
(d) IAP
Log (IP)
Soaking time (h)

FIG. 7

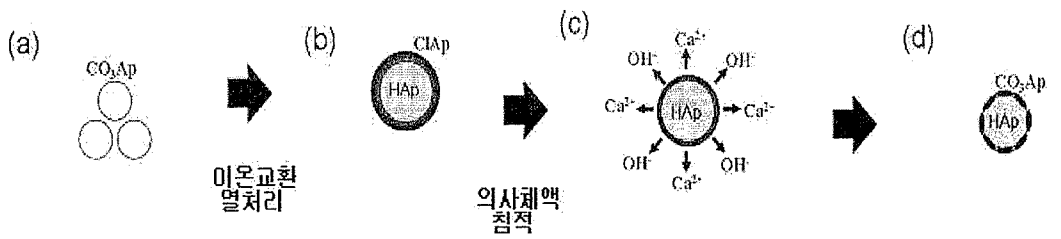


FIG. 8

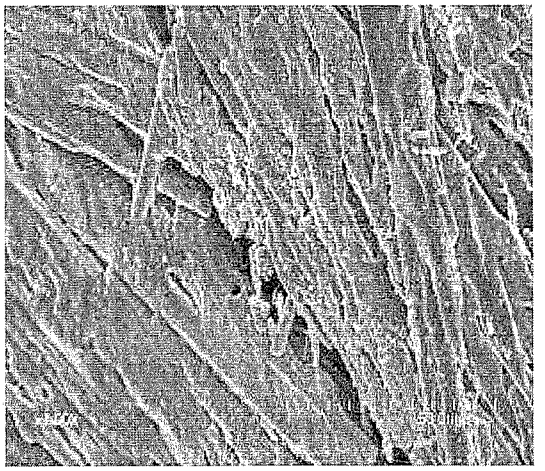


FIG. 9

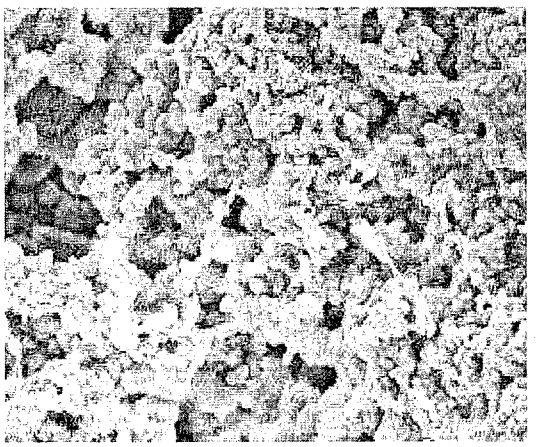


FIG. 10

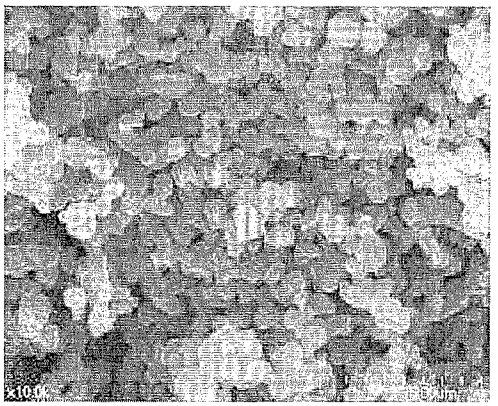


FIG. 11

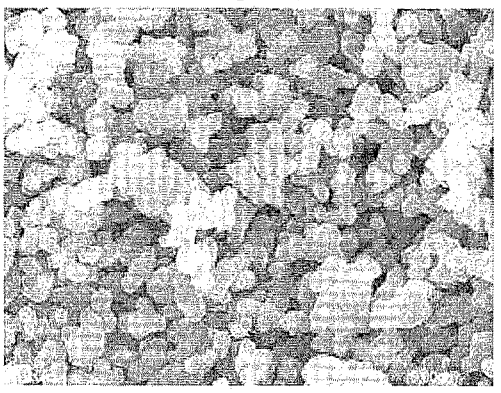


FIG. 12

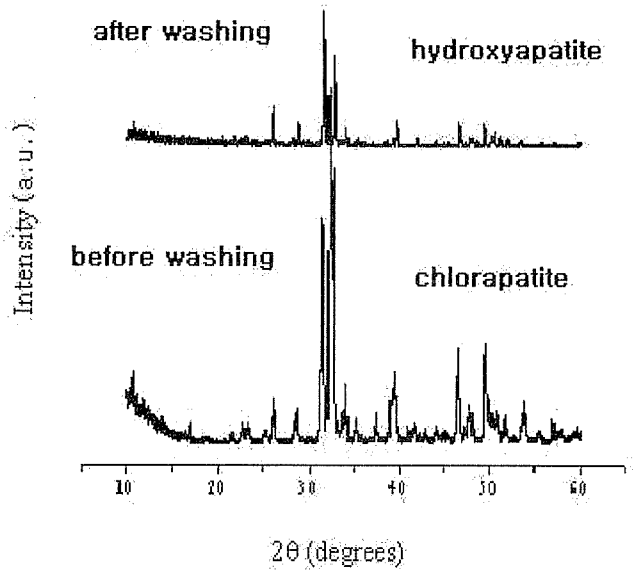


FIG. 13

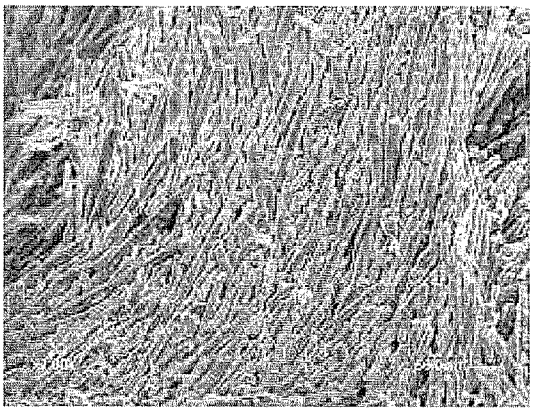


FIG. 14

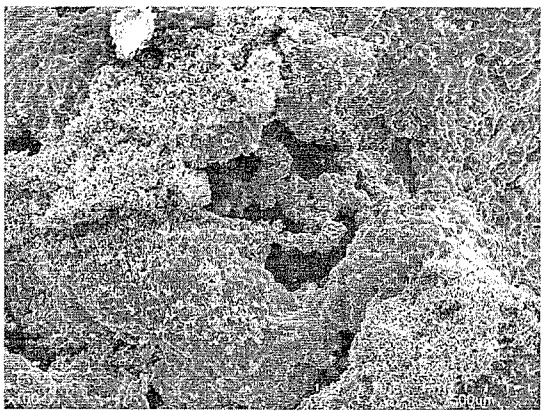
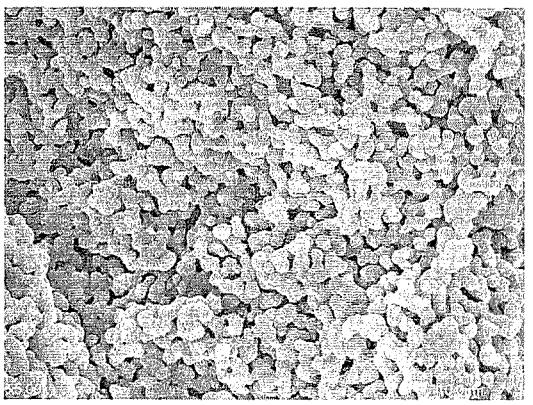


FIG.15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/001002

A. CLASSIFICATION OF SUBJECT MATTER

C01B 25/32(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 C01B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS(KPA, PAJ, FPD, USPATFULL) in KIPO, CA online & keywords: hydroxy-/chloro-apatite, bone, chloride ion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	KR 1020060099819 A (SEOUL NATIONAL UNIVERSITY INDUSTRY FOUNDATION) 20 SEPTEMBER 2006 See the abstract; claims 1-5, 7-9	10-13 1-9
A	KR 1020010048219 A (KIM, HYUNG GUN et al.) 15 JUNE 2001 See the abstract; claim 1	1, 4, 5, 8, 10, 11
A	KR1020000050294 A (CHOUNG, PILL HOON) 05 AUGUST 2000 See the abstract; claim 1	1, 4, 5, 8, 10, 11
A	JP10017310 A (EHIME PREFECTURE) 20 JANUARY 1998 See the abstract; claims 1, 3	1, 4, 5, 8, 10, 11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 MAY 2008 (26.05.2008)	**26 MAY 2008 (26.05.2008)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Jang, Ki Wan Telephone No. 82-42-481-5567

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2008/001002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR1020060099819A	20.09.2006	NONE	
KR1020010048219A	15.06.2001	AU200118990A1	04.06.2001
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JP10017310A	20.01.1998	JP10017310A2	20.01.1998