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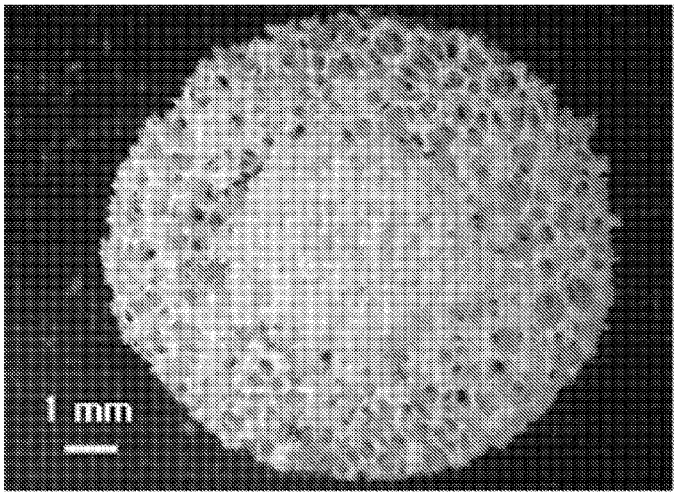
(54) **Title**: METHOD OF PREPARING CERAMIC/POLYMER COMPOSITE SCAFFOLDS WITH BIOACTIVE MOLECULES FOR HARD TISSUE REGENERATION

FIG. 1A



(57) **Abstract**: The present invention relates to a new organic/inorganic composite scaffold containing bioactive molecules and a ceramic/polymer composite scaffold having one or multiple bioactive molecules and a method for preparing the same. The main architecture of the novel composite scaffold is composed of an inorganic ceramic scaffold with one or a plurality of hole(s) in which a biocompatible and biogradable polymer containing bioactive materials is incorporated. Since the organic/inorganic composite scaffold containing bioactive molecules according to the present invention is made of a ceramic as the main frame, it can utilize its mechanical property essential for in vivo adaption and superior bioactivity. In addition, the present invention can release bioactive molecules from a biogradable polymer incorporated in the hole in the ceramic. Thus, the present invention can maximize bioaffinity and response whereby it can be useful in tissue regeneration and drug delivery.

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Title: METHOD OF PREPARING CERAMIC/POLYMER COMPOSITE SCAFFOLDS WITH BIOACTIVE MOLECULES FOR HARD TISSUE REGENERATION

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a new organic/inorganic composite scaffold in which a ceramic with bioactivity and superior mechanical properties and a polymer that is biogradable and contains bioactive molecules are incorporated. More specifically, the present invention relates to a method of preparing a ceramic/polymer composite scaffold for tissue generation, composed of a porous or nonporous ceramic scaffold with one or a plurality of hole(s) in which a biocompatible and biodegradable polymer containing bioactive molecules capable of more effectively inducing tissue generation, is simply and effectively incorporated, and a ceramic/polymer composite scaffold prepared by the same method. The present invention, when implanted in the body, can actively regenerate tissues by utilizing the advantages of both ceramics and polymers.

[0002] Because of their superior bioactivity and good mechanical property, bioceramics for *in vivo* implant, up to the present, have been the most widely used bioactive materials in dentistry, surgery, and plastic surgery for regenerating and treating bone loss and damage. Bioceramic materials are commonly divided into calcium phosphate ceramics, bioactive glasses, alumina, zirconia, and composites thereof. Calcium phosphate ceramics include hydroxyapatite (HA), tricalcium phosphate (TCP), tetracalcium phosphate (TTCP), dicalcium phosphate (DCPA), etc. For bioactive glasses, there are silica-based glasses, phosphate-based glasses, glass ceramics, and the like. When such bioactive ceramics are to be used as bone substitute implants or bone formation scaffolds, in order to successfully function as bone graft substitutes, they have to

possess osteoconductivity which allows for bone ingrowth and osteoinductivity by which the stem cells of a host tissue are encouraged and directed to induce the regeneration and recovery of bones. In addition, they should be capable of inducing the differentiation of stem cells into bone formation cells at various stages during bone formation or bone treatment, and should not be prone to structural failure. However, most currently available materials fail to satisfy such requirements. In particular, calcium phosphate based HA of which human bone is largely composed has a close chemical resemblance to bone, i.e., has a molecular ratio of calcium to phosphorus being 1.67. Although it is certainly advantageous in terms of the osteoconductivity, such as adhesion, diffusion, proliferation, etc., of bone cells due to its excellent bioactivity, it has been recently reported that HA has not exhibited osteoinductive capability which directly induces the formation of new bones. Accordingly, bioceramic materials including HA need to evolve from their prior passive function to generate bones to an active one, whereby upon *in vivo* implantation, the interaction between cells and biomaterials can be improved, resulting in a reduced period of treatment and a higher rate of success. In the bioceramic field, scaffolds with high interconnected high porosities, the form of which is block or granulate, have been extensively studied. Recently, scaffolds consisting of organic/inorganic composites (or hybrids) which can be combined with biogradable polymers are being studied. Nonporous ceramics are usually prepared by placing ceramic powders in a suitable container and pressing the powders under a specific pressure, and then sintering the pressed powders at a temperature in the range of 600 to 1600°C. As a representative porous ceramic preparing method, a polymeric sponge-template method has been known to make the most structurally ideal scaffolds. In that process, ceramic powders are dispersed in distilled water to prepare slurry having a specific viscosity. The slurry is then applied on the surface of a porous sponge having a web-like structure and the sponge is burned out by sintering to obtain a porous ceramic scaffold with web-like interconnected porous structures that replicate the form of a sponge (S. Ho et al., U.S. patent

application, Bi-Layered Bone-Like Scaffolds, PCT/US2008/072686, August 8, 2008). The polymeric sponge-template method is advantageous in terms of its ease in controlling the size and density of the pores and is capable of preparing various forms of ceramic scaffolds in a quick and simple manner. However, the sintered ceramic, unlike polymers, is not easy to surface modify and thus, it is difficult to provide various bioactive molecules which induce bone tissues. In order to solve the above problem, efforts have been made in developing ceramic/polymer organic/inorganic composite scaffolds such as PLGA/HA composite scaffolds (P. Zhang et al, *Biomaterilas*, 3, 58, 2009), chitosan/HA composite scaffolds (J. M. Oliveira et al., *Biomaterials*, 27, 6123, 2006), HA/polyurethane composite scaffolds (L. Wang et al., *Biomed. Mater.*, 4, 1, 2009), and the like. However, it is not suitable to utilize the bioactivity of ceramics in composites that are made mainly of polymers. In addition, composites have a lower strength than that of pure ceramics and they may elicit an inflammatory response. As such, their application is limited. Accordingly, there is a serious need to develop a novel organic/inorganic composite scaffold for more effectively regenerating and repairing bones than conventional materials, particularly, a composite scaffold combining the advantages of polymers and ceramics, i.e., superior bioactivity and mechanical strength, and is easy to process in providing bioactive molecules and biogradability.

[0003] Biodegradable synthetic polymers that are currently being used include polyglycolic acid (PGA), polylactic acid (PLA), poly(D,L-lactic-o-glycolic acid) (PLGA), poly-ε-caprolacton (PCL), poly(lactic acid-ε-caprolacton) (PLCL), polydioxanone (PDO), polytrimethylenecarbonate (PTMC), poly(amino acid), polyethyleneoxide, pluronic, polyanhydride, polyorthoester, and copolymers thereof. For natural polymers, there are bovine serum albumin, collagen, gelatin, chitin/chitosan, alginate, hyaluronic acid, chondroitin sulfate, fibrinogen, cellulose, heparin, dextran, and copolymers thereof. Up until now, a few synthetic polymers such as PGA, PLA, PLGA, and pluronic, and most natural polymers such as chitosan,

etc., have been used as microspheres for drug delivery and porous polymer scaffold materials for *in vivo* human tissue regeneration, which have been approved as biocompatible polymers from the U.S. Drug and Food Association.

[0004] As representative techniques to prepare a scaffold for tissue generation using the synthetic and natural polymers as above, there are a solvent-casting and particulate-leaching technique in which mono-crystalline salts are added in a solvent and the solution is then dried to cast salts (A. G. Mikos et al., *Polymer*, 35, 1068, 1994), a gas foaming technique in which a polymer is swollen using a CO_2 gas (L. D. Harris et al., *J. Biomed. Mater. Res.*, 42, 396, 1998), a fiber extrusion and fabric forming process in which nonwoven fabrics are prepared into a polymeric mesh (K. T. Paige et al., *Tissue Engineering*, 1, 97, 1995), a thermally induced phase separation technique in which a solvent contained in a polymer solution is dipped in a non-solvent to produce pores (C. Schugens et al., *J. Biomed. Mater. Res.*, 30, 449, 1996), an emulsion freeze-drying technique in which a polymer solution is mixed with water to prepare the same into an emulsified solution and the prepared emulsion is then frozen with liquid nitrogen and dried (K. Whang et al., *Polymer*, 36, 837, 1995), and the like. The polymer scaffold prepared as above may be provided with bioactive molecules by mixing an organic or aqueous solution containing bioactive molecules with a polymer solution or directly mixing particles of the bioactive molecules with the same (J. J. Yoon et al., *Biomaterials*, 24, 2323, 2003). Another approach to provide bioactive molecules is to fix bioactive molecules on the surface of a prepared scaffold through surface modification (J. S. Son et al., *Tissue Eng. Regen. Med.*, 5, 528, 2008). The scaffolds prepared according to the techniques described above may induce relatively superior tissue generation due to their high porosities and release of bioactive molecules. However, because of their low mechanical properties, the above scaffolds are not suitable for hard tissue regeneration and, after *in vivo* implantation, an undesirable inflammatory response may be elicited when the polymer decomposes. As such, the applications are limited.

[0005] The present inventors made a large effort in preparing an organic/inorganic composite scaffold for hard tissue regeneration combining the advantages of ceramics and polymers. As a result, the present inventors found that if a porous or nonporous ceramic scaffold has one or a plurality of hole(s) and a polymer containing bioactive molecules is combined into said hole(s) in various forms such as blocks, films, fibers, nonwoven fabrics, gels, etc., the polymer combined in the hole(s) decomposes without any deformation because of the high mechanical strength of the ceramic while retaining the bioactivity and at the same time the bioactive molecules are continuously released, thereby capable of inducing tissue regeneration more effectively.

SUMMARY OF THE INVENTION

[0006] It is an objective of the present invention to prepare an organic/inorganic scaffold for use in a scaffold for hard tissue regeneration and a drug delivery system, in which a ceramic and a polymer containing bioactive molecules are combined. The present invention is capable of controlling the size, density, and the porosity of the ceramic that mainly builds the present composite scaffold, as well as the shape and size of the ceramic scaffold to be prepared. Further, one or a plurality of holes can be created in a ceramic scaffold so as to penetrate the same using a simple method and in this hole(s), a polymer(s) containing various kinds of bioactive molecules, which is prepared in various forms, can be incorporated whereby multiple bioactive molecules are continuously released from the polymer(s) while, due to the ceramic's mechanical property, retaining the shape without any deformation during the period necessary for tissue generation. As such, the present invention can solve problems in pure ceramics, i.e., the difficulty in giving bioactive molecules that are more advantageous in tissue regeneration, and problems in pure polymers, i.e., the ease in providing bioactive molecules but the low mechanical strength and the possibility of eliciting an inflammatory response when decomposed.

Therefore, it is an objective of the present invention to provide a method of preparing an organic/inorganic composite scaffold more simply and more effectively.

[0007] In order to achieve the objectives described above, the present invention provides:

1) preparing a porous or nonporous ceramic scaffold having one or plurality of hole(s);

2) dissolving a biogradable polymer in an organic solvent or aqueous solution and then dissolving the solution together with bioactive molecules to prepare a polymer solution containing bioactive molecules;

3) placing a polymer into the hole(s) of the ceramic scaffold, where the polymer is obtained by directly or externally solidifying the polymer solution containing bioactive molecules in the holes within the ceramic structure or where the bioactive molecules are fixed on the surface of the polymer through surface modification; and

4) forming apatite on the surface of the organic/inorganic composite material through biomimic processing.

[0008] The present invention also provides a scaffold for tissue regeneration prepared according to the method described above and a ceramic/polymer composite scaffold that can be used as a drug delivery system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **FIGS. 1A, 1B,** and **1C** are photographs of an apatite-coated PLA composite porous scaffold containing HA/bioactive molecules prepared according to the present invention, observed using a stereoscope, a micro CT, and a scanning electron microscope (SEM), respectively.

[0010] **FIGS. 2A** and **2B** are photographs of a ceramic/polymer composite scaffold containing bioactive molecules prepared according to the present invention, observed using a

stereomicroscope.

[0011] **FIGS. 3A, 3B,** and **3C** show the drug release behavior of dexamethasone released from an apatite-coated PLA composite porous scaffold containing HA/bioactive molecules prepared according to the present invention scaffold and compare the composite scaffold to Comparative Example 1 with respect to cellular specificity.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention relates to a method to effectively provide bioactive molecules to ceramics and to a novel form of a ceramic/polymer composite scaffold. The ceramics used as the main substrate of the composite can be prepared in porous or nonporous forms and scaffolds obtained from the prepared ceramics have a structure in which one or a plurality of holes penetrate the scaffolds. In ceramic hole(s), natural or synthetic polymers containing bioactive molecules are incorporated and the polymers can be prepared into various forms such as block, film, fiber, nonwoven fabric, particles, gels, etc. In addition, since bioactive molecules can be incorporated in ceramics by directly mixing with a polymer or fixation on the surface of the polymer through surface modification, the amount and release rate of bioactive molecules can be controlled and at the same time polymers containing different bioactive molecules can be incorporated into a plurality of holes in the ceramic. As such, it is possible to prepare ceramic/polymer composite scaffolds capable of containing multiple bioactive molecules.

[0013] Hereinafter, the present invention will be described in detail in a step by step manner.

[0014] The ceramic/polymer composite scaffold preparation method according to the present invention comprises the following steps of:

1) preparing a porous or nonporous ceramic scaffold having one or a plurality of hole(s);

2) dissolving a polymer in an organic solvent or water solution to prepare a polymer solution and

then adding or suspending bioactive molecules in the polymer solution to prepare a polymer solution containing bioactive molecules;

3) placing a polymer in the hole in the ceramic scaffold, where the polymer is solidified from the polymer solution containing bioactive molecules inside or outside the hole, or has a surface in which bioactive molecules are fixed through surface modification; and

4) coating the surface of the organic/inorganic composite material with apatite through biomimic processing.

[0015] Step 1) is directed to a step of preparing a porous or nonporous ceramic scaffold having one or plurality of hole(s). The nonporous ceramic scaffold can be prepared by compressing ceramic powders in a desired form under appropriate pressure and sintering the compressed ceramic powders at a temperature in the range of 600 to 1600°C. The porous scaffold can be provided with hole(s) by mixing ceramic particles and pore forming materials to prepare a mixture, compressing the mixture, and sintering the compressed mixture, or a polymeric template method can be used. Specifically, a polymer sponge template method can be used in which a ceramic slurry is prepared in addition to a suitable binder, the prepared slurry is applied on porous polymer sponge having one or a plurality of pores, the covered polymer sponge is dried and sintered at a temperature in the range of 600 to 1600°C, thereby burning out the polymer sponge while obtaining a porous ceramic scaffold having the same shape as the polymer sponge used.

[0016] Ceramics suitable for the present invention include any ceramics that can be used for *in vivo* implant calcium phosphate based ceramics and bioactive glass, zirconia, alumina, silica., and composites thereof. Suitable ceramics include, but are not limited to, hydroxyapatite (HA), tricalcium phosphate (TCP), tetracalcium phosphate(TTCP), dicalcium phosphate (DCPA), zirconia, alumina, glass ceramic, and composites thereof, and silica/glass and silica/calcium phosphate based composites, etc.

[0017] As a pore forming material which can be used for the preparation of the porous ceramic of the present invention, any materials which can be removed by thermal treatment can be used, for example synthetic or natural polymer compounds or inorganic materials, and mixtures thereof. Representative pore forming materials may include polymers containing an ester group such as polyester (PET), etc., polymers containing an acryl group such as polymethylmethacrylate (PMMA), etc., polymers containing a vinyl group such as polyvinylalcohol (PVA), etc., polymers containing a urethane group such as polyurethane (PU), polymers containing an amide group such as nylon (NY), etc., polymers containing an imide group such as polyimide (PI), polymers containing a cellulose group such as carboxymethylcellulose (CMC), etc., rubbers such as epoxy resins, SBR, etc., and composites thereof or thermoplastic or thermosetting resins obtained from copolymerization thereof, and sucrose, stearic acid, dextrin, flour, chitosan, alginic acid, gelatin, starch, hydrogen peroxide compounds, carbonate compounds, etc., and particles or fabrics of composites thereof. Among the materials mentioned above, thermosetting materials having a porous structure such as urethane, silicone, synthetic and natural rubber series sponge, more specifically sponge obtained from polyurethane or polyesterurethane copolymers, may be used, but are not limited thereto.

[0018] In fabricating the ceramic slurry, in order to enhance the sintered stability and actuation performance of ceramic particles, a binder can be added. The binder may, for example, include CMC, PVA, starch, sodium silicate, polyvinyl butyl, methacrylate, polyacrylate, polyacrylic acid, polyethylene glycol, etc. In addition, a dispersant for improving the dispersion of ceramic particles and a drying agent to prevent cracking which may occur during the drying of the coated sponge can be added. It is desirable to use as a binder PVA and CMC in the range of 0.1 to 5%, as a dispersant and a drying agent ammonium polyacrylate (APA) and N, N-dimethylformamide (DMF) in the range of 0.1 to 5%.

[0019] Suitable hole(s) which penetrates a ceramic scaffold may have a size ranging from

several millimeters to several centimeters. The hole is made in a physical manner such as a drill, etc., during the post-ceramic scaffold processing. Alternatively, before the ceramic scaffold processing, materials capable of providing pores can be combined or a ceramic scaffold having holes can be prepared by sintering a polymer sponge template that has been given holes. Specifically, before applying ceramic particles on a polyurethane sponge, hole(s) of a desired size and shape are given to polyurethane sponge. The hole(s) can be prepared as one hole with a size of several millimeters to several centimeters or several holes with various sizes. Specifically, the hole(s) has a diameter in the range of 0.1 mm to 50 mm and in a number of from 1 to 10.

[0020] Step 2) is directed to preparing a polymer solution containing bioactive molecules by dissolving or suspending a biogradable polymer together with bioactive molecules in an organic solvent or water solution suitable for dissolving a biogradable polymer.

[0021] As biogradable polymers which can be used in the present invention, any nontoxic polymer that can be *in vivo* decomposed can be used. Such polymers may include polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolide), poly-ε-caprolacton (PCL), polyamino acid, polyanhydride, polyorthoester, and derivatives and copolymers thereof; collagen, gelatin, chitin/chitosan, alginate, albumin, hyaluronic acid, fibrinogen, cellulose, dextrin, pectin, polylysine, protein derivatives, and copolymers thereof. Among the polymers mentioned above, it may be desirable to use polymers that are approved as a biocompatible and biodegradable polymer from the U.S. Food and Drug Association, e.g., polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolide) (PLGA), or mixture thereof, natural polymer and mixtures thereof. The biogradable polymer may have, but is not limited to, a weight average molecular weight of 500 to 2,000,000 g/mol, more specifically 10,000 to 700,000 g/mol.

[0022] Solvents used for dissolving the biogradable polymer described above in the present invention may include, but may be different according to the kind of the polymer, water,

hydrochloric acid, acetic acid, methylenechloride, chloroform, carbon tetrachloride, acetone, dioxane, tetrahydrofuran, hexafluoroisopropanol, etc. Among the solvents mentioned above, water, acetic acid, methylenechloride, and chloroform may be used. In such solvents, a biogradable polymer is dissolved in combination with bioactive molecules, or only a biogradable polymer is dissolved to make a polymer solution and then bioactive molecules are suspended in the polymer solution, to prepare a polymer solution containing bioactive molecules. The biogradable polymer is 0.01 to 15% by weight based on the solvent and the bioactive molecules can be added in an amount of 10^{-7} to 50% by weight based on the biogradable polymer.

[0023] Bioactive molecules that can be used in the present invention are growth factors, growth hormones, peptides or protein medical products, anti-inflammatory drugs, anti-cancer agents, antiviral agents, sex hormone, antibiotics, antifungal agents, or compounds. Such bioactive molecules may include, for example growth factors such as transforming growth factor (TGF), fibroblast growth factor (FGF), bone morphogenic protein (BMP), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), placental growth factor (PIGF), granulocyte colony stimulating factor (G-CSF), etc.; peptide or protein medical products such as heparin, animal growth hormones, human growth hormones (hGH), erythiopoietin (EPO), interferon (INF), follicle stimulating hormone (FSH), progesterone (LH), luteinizing hormone-releasing hormone (LH-RH) agonists such as goserelin acetate, leuprolid acetate, triptoneline acetate, etc.; anti-inflammatory drugs such as dexamethasone, indomethacin, ibuprofen, ketoprofen, piroxicam, flurbiprofen, diclofenac, etc.; anticancer agents such as paclitaxel, doxorubicin, carboplatin, camptothecine, 5-fluorouracil, ciproretin, cytosine arabinose, methotrexate, etc.; antiviral agents such as acyclovir, etc.; sex hormones such as testostherone, estrogen, progestheron, estradiol, etc.; antibiotics such as tetracycline, minocycline, doxycycline, ofloxacin, levofloxacin, ciprofloxacin, clarithromycin, erythromycin, cefaclor, cefotaxime,

imipenem, penicillin, gentamycin, strentomycin, vancomycin, etc.; antifungal agents such as ketoconazole, itraconazole, fluconazole, amphotericin-B, griseofulvin, etc.; and other compounds such as β-glycerophosphate, ascorbate, hydrocortisone, 5-azacytidine, etc.

[0024] Step 3) is directed to placing a polymer in the hole in the ceramic scaffold prepared in Step 1), where the polymer is solidified from the polymer solution containing bioactive molecules prepared in step 2) above inside or outside the hole, or has a surface in which bioactive molecules are fixed through surface modification. First, as a method to solidify polymer containing bioactive molecules directly in a ceramic scaffold, a composite scaffold in which a porous polymer is incorporated in the ceramic can be prepared by mixing a certain amount of an effervescent material (porogen) capable of forming pores on a polymer with a polymer to make polymer/effervescent mixture, placing the mixture in the hole of the ceramic scaffold prepared in Step 1) for solidification thereof, and then adding the solidified mixture in an effervescent medium to boil (Method A). The porous scaffold in a block form according to the present invention is prepared by using, but not limited to, a salt casting/gas foaming method.

[0025] The effervescent mixture used for pore formation in the present invention is composed of carbonates and organic acids. Specifically, the effervescent mixture is not only a non-toxic material that can be used in general drugs, but can also be easily dissolved in water and in a solid state having a specific size. Such carbonates may include salts generating carbon dioxide and oxygen such as sodium hydrogen carbonate, sodium carbonate, ammonium hydrogen carbonate, potassium bicarbonate, potassium carbonate, calcium carbonate, peroxide hydrogen compounds, and the like. As organic acids, citric acid, tartaric acid, succinic acid, maleic acid, fumaric acid, malonic acid, malic acid, gluconic acid, mucic acid, amino acids, etc. can be used.

[0026] Alternatively, a polymer/effervescent mixture containing bioactive molecules are placed in a suitable container not in a ceramic scaffold for solidification thereof. Thereafter, the solidified mixture is added in an effervescent medium to boil the effervescent material and the

obtained polymer is cut into a size tailored to a hole and the cut polymer is placed into the hole (Method B).

[0027] In addition, a polymer containing bioactive molecules is prepared into films, fibers, nonwoven fabrics, particles, and gels and whichever form prepared can be placed into the ceramic scaffold (Method C). Through surface treatment, the bioactive molecules presented in Step 2) can be electrically combined to the surface of the polymeric form prepared above or to hydrophilic monomers containing functional groups. The bioactive molecules combined polymer form can be placed into the hole in the ceramic scaffold (Method D).

[0028] The porous polymer in a block form which can be used in Method A) above is used with an effervescent mixture having a diameter of 0.005 to 1 cm in a ratio of 5:1 to 20: 1, based on the weight of the biogradable polymer. The porous polymer is specifically in the form of blocks in which pores with a porosity of 5 to 98% and a size of 0.1 nm to 5 mm are interconnected.

[0029] Subsequently, the polymer/effervescent mixture prepared above is placed into the ceramic hole having a diameter in the range of 1 to 50 mm and allowed to evaporate the solvent at -196°C to room temperature. The mixture boils in an effervescent medium and is then dried to prepare a polymer/ceramic scaffold containing bioactive molecules. After the completion of the foaming process above, an excessive amount of water contained in the porous scaffold is removed. In order to minimize the shrinking of the porous scaffold due to a rapid evaporation of a very small amount of an organic solvent remaining in the porous scaffold, it may be freeze-dried or vacuum dried at room temperature or at a glass transition temperature (T_g) of the obtained polymer scaffold.

[0030] Method B is the same as Method A, only differing in that a polymer/effervescent mixture containing bioactive molecules is placed into a suitable container or mold, not in a ceramic hole, to solidify. The porous scaffold prepared thus is cut into a size tailored to a ceramic hole with a diameter between 1 and 50 mm or a polymer scaffold is prepared to have a

size tailored to the hole and the prepared polymer scaffold is placed. It may be desirable to use a polymer having bioactive molecules in the form of blocks in which pores with a porosity of 5 to 98% and a size of 0.1 nm to 5 mm are interconnected.

[0031] Polymer films, nonwoven fabrics, fibers, particles, gels, etc., containing bioactive molecules, which can be used in Method C, can be prepared into porous or nonporous types. Any form that can be placed into a ceramic hole can be used. For example, polymer films containing bioactive molecules can be prepared by solvent casting or melt-pressing and into several micrometers to several centimeters. The polymer films containing bioactive molecules prepared is thus cut or rolled into the ceramic hole, or pulverized into particles and placed into the hole in the form of particles. Specifically, films with a thickness of 0.001 mm to 1 mm, which are nonporous or have a porosity in the range 5 to 98%, are cut or molded and rolled into the ceramic hole.

[0032] Polymer nonwoven fabrics containing bioactive molecules can be prepared by electrospinning, melt-blown, etc., and cut or rolled, and placed into a ceramic hole. The diameter of fibers may be in the range of several nanometers to several millimeters and the thickness thereof may be in the range of several micrometers to several centimeters. Specifically, the fibers have a porosity in the range of 5 to 98%, where fiber aggregates having a diameter of 10 nm to 1 mm are placed into a ceramic hole. When the fibers are pressed into nonwoven fabrics, the thickness of the nonwoven fabrics is in the range of 10 μm to 50 mm and the nonwoven fabrics are cut or rolled, and placed into the ceramic hole.

[0033] Polymer particles containing bioactive molecules may have a diameter of several nanometers to several micrometers and can be prepared by any method to make polymer particles containing bioactive molecules such as water/oil or water/oil/water emulsification, spinning, phase separation, inter-polymeric polyelectrolyte complex, etc. The prepared particles can be spherical or powdery. Specifically, particles containing bioactive molecules are

placed into a ceramic scaffold hole, where particles have a diameter of 10 nm to 1 mm, and are nonporous or have a porosity of 5 to 98%.

[0034] As polymer gels containing bioactive molecules, among the polymers presented in Step 2, any polymer capable of gelation can be used. A polymer having a concentration of 0.1 to 90% is gelled at a temperature of 0 to 90□ and the polymer gel is molded into a size tailored to the ceramic hole and placed into the hole. Specifically, polymer gels having a porosity of 5 to 98% are molded above the temperature or concentration at which the polymer becomes a gel and placed into a ceramic hole.

[0035] In addition, the polymer blocks, films, fibers, nonwoven fabrics, particles, gels, etc., containing bioactive molecules mentioned above can be separately placed into a plurality of ceramic holes.

[0036] Method D) is a process in which a polymer is prepared into blocks, fibers, nonwoven fabrics, particles, etc., and the prepared polymer is surface-treated to fix bioactive molecules on the surface of the polymer. For example, a polymer film is prepared into a suitable size according to Method C) and if necessary, after performing an oxygen or argon plasma treatment, hydrophilic monomers containing functional groups are directly graft-polymerized on the surface of the scaffold, or the plasma-treated scaffold is immersed in a solution of hydrophilic monomers containing functional groups and subject to graft polymerization with heat treatment to modify the surface of the scaffold. On the modified surface of a polymer film, bioactive molecules can be fixed by chemically combining to the functional groups or by physically electrically combining to the same. The bioactive molecules-fixed film is molded into a suitable shape and placed into the ceramic hole.

[0037] Hydrophilic monomers containing a functional group used in the present invention may, for example, include organic acids containing a carboxy group in the terminal group such as acrylic acid, maleic acid, itaconic acid, cis-aconic acid, crotonic acid, fumaric acid, trans-flutanic

acid, etc., and mixtures of the foregoing. In addition, monomers having phosphates in the terminal group, e.g., vinylphosphonic acid, ethylene glycol methacrylate phosphate (EGMP), etc., and ionic polymers such as hyaluronic acid, heparine, condroithin sulfate, albumin, polylysine, chitosan, alginic acid, pectin, dextran sulfate, etc. and mixtures thereof may be used.

[0038] Step 4) is directed to coating the surface of the organic/inorganic composite material prepared in Step 3) above with apatite through biomimic processing which is not required. Specifically, the polymer containing bioactive molecules incorporated in the ceramic hole is PLA, PLGA, and PGA, which are al approved by the U.S. FDA, or copolymers thereof or if necessary, an apatite may be formed on the surface of a natural polymer or ceramic. First, the ceramic/polymer composite containing bioactive molecules prepared above is dipped in a simulated body fluid (SBF) at 37± 0.5□ and at a pH of 6.4 to 7.4 for 1 hour to 30 days. A SBF solution is obtained by adding in 1ℓ distilled water, 8.035 g NaCl, 0.355 g $NaHCO_3$, 0.225 g KCl, 0.231g $K_2HPO_4 \cdot 3H_2O$, 0.311 g $MgCl_2 \cdot 6H_2O$, 0.292 g $CaCl_2$, 0.072 g Na_2SO_4, 6.118 g $[(HOCH_2)_3(CNH_2)]$, and 39 □1.0 M HCl and the obtained solution is specifically used in a one- to five-fold concentration.

[0039] In addition, in order to form apatite on the surface of a ceramic/polymer composite quickly, an alternate dipping process can be conducted in which the ceramic/polymer composite is dipped once to five times in a 1 to 90% ethanol solution where calcium chloride ($CaCl_2$) and dipotassium phosphate (K_2HPO_4) are dissolved to form a precursor of the apatite. Specifically, the following cycle is repeated three times: 0.1 M calcium chloride and potassium phosphate are dissolved in a 50% ethanol solution. First, the ceramic/polymer composite is dipped in an ethanol solution containing 0.1 M calcium chloride for 10 seconds, followed by dipping in a pure 50% ethanol solution for 1 second, and then dried at room temperature for 3 minutes. The ceramic/polymer composite undergoing the above is then dipped in an ethanol solution containing 0.1 M dipotassium phosphate for 10 seconds, followed by dipping in a pure 50%

ethanol solution for 1 second, and then dried at room temperature for 3 minutes.

[0040] In fabrication of the ceramic/polymer composite body containing bioactive molecules prepared above, the ceramic is nonporous or has porosity in the range of 5 to 98% and the pore distribution and the size thereof are proportional to the amount or the pore formation material used or pores distributed on the polymer sponge per inch. In addition, a hole penetrating the ceramic has a diameter in the range of 0.1nm to 50 mm and the number of the hole is in the range of 1 to 10. The polymer containing bioactive molecules is nonporous or has porosity in the range of 5 to 98%. In addition, the pore size of the polymer is in the range 0.1 nm to 5 mm and any form that can be placed into a hole in the ceramic, e.g., blocks, films, fibers, nonwoven fabrics, particles, gels, may be possible. The preparation method according to the present invention gives a ceramic scaffold hole(s), by which a polymer containing bioactive molecules can be directly solidified in the hole(s) or a polymer solidified outside the hole can be placed in the ceramic hole(s). Thus, the processing is simple and incorporation of multiple bioactive molecules in a plurality of holes in the ceramic is possible. In addition, since the present invention can be placed into a ceramic material in a desired form while retaining the bioactivity of the ceramic material, various kinds of bioactive molecules can be incorporated in a polymer. Further, stable and continuous drug release can be anticipated from the above and thus the present invention can be more effectively used as a scaffold for hard tissue regeneration and control-released drug delivery system.

[0041] Hereinafter, the present invention will be explained more in detail with reference to the working examples below. However, the following examples are for illustrative purposes only and the invention is not intended to be limited by these examples.

EXAMPLE 1

[0042] A PLA composite scaffold containing hydroxyapatite (HA)/dexamethasone (DEX)

(calcium phosphate ceramics) was made as follows. First, 5 g of nano-sized HA powders was dispersed in distilled water. As a binder, a dispersant, and a drying agent, PVA, CMC, APA, and DMP were added in the dispersion in an amount of 3%, 3%, 7%, 5% based on the HA powders, which was continually evaporated while stirring at 90°C to prepare a HA slurry. Thereafter, a 60 ppi polyurethane foam was cut to have a diameter of 13 mm and a height of 7 mm. The cut sponge was given a hole with a diameter of 7 mm and then coated with the HA slurry prepared above. The coated sponge was dried at room temperature for 24 hrs and then sintered at 1230□ for 3 hours. The sintered sponge was coated with the same HA slurry again and then sintered again finally to obtain a HA porous scaffold with a diameter of 10 mm and a height of 5 mm, and a hole with a diameter of 5 mm.

[0043] Subsequently, 600 mg PLA (IV: 0.55-0.75 dL/g) was completely dissolved in 3.4 mL dichloromethane and dispersed with the addition 50 mg dexamethasone until it becomes homogenous. Thereafter, 8g sodium percarbonate (SPC) was added in the dispersion as a pore forming material to prepare a PLA/SPC mixture containing dexamethasone. The PLA/SPC mixture thus prepared was placed into the hole having a diameter of 5 mm of the ceramic scaffold prepared above and vacuum-dried at room temperature. In order to remove SPC from the dried composite, the mixture was added in a 1% catalase (hydrogen peroxide decomposing enzyme) solution while stirring for 48 hours. The solution was again stirred for 24 hours while refreshing distilled water several times and peroxide remaining in the scaffold was completely removed. Finally, a HA/PLA composite scaffold having a 5 mm hole in which PLA containing dexamethasone was incorporated was obtained.

[0044] An apatite coating was applied on the surface of the PLA composite scaffold containing HA/dexamethasone prepared above with oxygen plasma treatment and using SBF. First, the prepared composite body was placed in a plasma generating chamber with an inflow of oxygen and the chamber was treated at 200 mtorr at 30 watt for 3 minutes. In order to provide a

precursor capable of quick apatite formation on the surface of PLA, an alternate dipping process was repeated three times in which the composite body was dipped in a 30% ethanol solution containing 100 mM calcium chloride for 10 seconds, followed by dipping in a pure 30% ethanol solution for 1 second, and dried at room temperature for 3 minutes, and the composite body was then dipped in a 30% ethanol solution containing 100 mM dipotassium phosphate for 10 seconds, followed by dipping in a pure 30% ethanol solution for 1 second, and dried at room temperature for 3 minutes. After the completion of the above process, the scaffold was dipped in a SBF solution with a five-fold ionic concentration and stirred at a rate of 100 rpm at 37°C for 24 hours finally to obtain an apatite-coated PLA porous composite scaffold containing HA/dexamethasone.

[0045] The surface and cross-section of the composite prepared above were examined using a stereomicroscope, a scanning electron microscope (SEM), helium pycnometry, a micro CT, etc. The results are illustrated in **FIGS. 1A**, **1B**, and **1C**. In the HA/PLA porous composite thus prepared, HA and PLA were found to have a pore size of 350 to 500 μm and 100 to 700 μm, respectively. The composite also exhibited an at least 95% porosity. Further, HA and PLA were well interconnected with no evidence of cracks at their interface and they were observed to form a uniform apatite coating on the surface and the cross-section.

EXAMPLE 2

[0046] HA and tricalcium phosphate (TCP) powders were mixed in a ratio of 50 to 50 to make a slurry. According to the same procedure as in Example 1, a HA/TCP ceramic porous scaffold was prepared. PLGA (75:25, IV: 0.16-0.24 dl/g) was adjusted to have the same concentration as in Example 1 and then mixed with 8 g salt (NaCl) to obtain a mixture. The obtained mixture was placed in a silicone mold (diameter: 5 mm, height: 5 mm) and quickly frozen with liquid nitrogen, followed by removing the salt with distilled water to prepare a porous PLA containing dexamethasone. The prepared porous PLA containing dexamethasone was placed into a 5 mm

ceramic hole and according to the same procedures as in Example 1, an apatite-coating PLGA composite scaffold containing HA/TCP/dexamethasone was obtained.

[0047] The composite scaffold thus prepared showed results similar to those obtained in Example 1 with respect to the shape, and the pore size and distribution.

EXAMPLE 3

[0048] TCP powders were placed in a compressing holder (diameter: 13 mm, height: 7 mm) and compressed under a pressure of 10 Mpa. The compressed powders were sintered at 1230□ to obtain nonporous TCP ceramic scaffold disks. An apatite-coated PLA composite scaffold containing TCP/dexamethasone was prepared according to the same procedure as in Example 1 except that the prepared TCP scaffold was given a 5 mm hole(s) using a drill.

[0049] The composite scaffold thus prepared showed results similar to those obtained in Example 1 with respect to the shape, and the size and distribution of pores in PLA.

EXAMPLE 4

[0050] A slurry obtained by mixing HA and TCP powders in a ratio of 75 to 25 was applied on 60 ppi polyesterurethane foam. According to the same procedures as in Example 1, a HA/TCP ceramic scaffold with a diameter of 10 mm and a height of 5 mm, and a hole diameter of 5 mm, was made. Thereafter, 1 g PLGA (85:15, IV: 1.3-1.7 dl/g) was dissolved in 3 mL chloroform and a PLGA film having a thickness of 300 μm was prepared using a solvent casting method. The prepared film was subject to plasma treatment using argon gas and acrylic gas such that the acrylic acid was attached to the PLGA film. Subsequently, carboxy groups in the acrylic acid attached on the surface of PLGA were activated using N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carboimide hydrochloride (EDC) and then reacted with addition of a certain amount of heparin to obtain a PLGA film having a heparin-attached surface.

The PLGA thus prepared was rolled and placed into the 5 mm hole of the HA/TCP ceramic prepared above. Finally, a HA/TCP/heparin-fixed PLGA film composite scaffold was obtained.

[0051] In the composite scaffold thus prepared, the HA/TCP ceramic porous scaffold achieved characteristics similar to those obtained in Example 1 and it was observed that the PLGA film having a heparin-fixed surface was well incorporated in the ceramic hole.

EXAMPLE 5

[0052] A slurry obtained by mixing HA and TCP powders in a ratio of 25 to 75 was applied on 100 ppi polyurethane foam and according to the same procedure as in Example 1, a HA/TCP ceramic porous scaffold with a diameter of 2 cm and a height of 1 cm, and a hole diameter of 5 mm, was prepared. As described in Example 2, a porous PLA scaffold in the form of blocks was prepared using PLA (IV: 0.8-1.2 dL/g) and as described in Example 4, heparin was fixed on the surface thereof. The porous PLA scaffold was then immersed in an aqueous solution containing 10 μg/mL bone morphogenic protein (BMP) to prepare a heparin and BMP-fixed porous PLA through ion complex, which was then placed into the 5 mm hole in the HA/TCP ceramic scaffold finally to obtain a HA/TCP/BMP fixed PLA porous composite scaffold.

[0053] The composite scaffold thus prepared achieved results similar to those in Example 1, only differing in that the pore size of HA/TCP was 150 μm in average.

EXAMPLE 6

[0054] A slurry obtained from tetracalcium phosphate (TTCP) powders was applied on a 80 ppi polyurethane sponge and according to the same procedures as in Example 1, a TTCP ceramic scaffold with a diameter of 10 mm and a height of 5 mm, and a hole diameter of 5 mm, was prepared. 100 g PLGA (70:30, IV: 2.0-2.8) was mixed with 1 g dexamethasone to obtain nonwoven fabrics with a thickness of 500 μm by a melt-blown method. Subsequently, the

nonwoven fabrics were cut into a size tailored to a 5 mm hole in TTCP and rolled into the hole. As described in Example 1, an apatite coating was formed on the surface of the nonwoven fabrics finally to obtain apatite coated PLGA composite scaffold nonwoven fabrics containing TTCP/dexamethasone.

[0055] In the composite scaffold thus prepared, it was observed that the TTCP ceramic porous scaffold has an average pore size of 250 μm and that the prepared nonwoven fabrics were well incorporated in the hole of the ceramic scaffold.

EXAMPLE 7:

[0056] A slurry obtained from HA powders was applied on a 80 ppi polyurethane foam and according to the same procedures as in Example 1, a HA ceramic scaffold with a diameter of 10 mm and a height of 5 mm, and a hole diameter of 5 mm, was made. As described in Example 6, a PLGA fiber was made and then placed into the 5 mm hole of the HA ceramic prepared above without being compressed. Finally, an apatite-coated PLGA fiber composite scaffold containing HA/dexamethasone was obtained.

[0057] In the composite scaffold thus prepared, as shown in **FIG. 2A**, it was observed that the fiber aggregates were well incorporated in the hole of the ceramic scaffold.

EXAMPLE 8

[0058] A slurry obtained from DCPA powders was covered onto a 60 ppi polyurethane sponge and according to the same procedure as in Example 1, a DCPA ceramic scaffold with a diameter of 10 mm and a height of 5 mm, and a hole diameter of 4 mm, was made. Poly ε-caprolactone (PCL) was dissolved in chloroform to make a 10% by weight solution. The solution was then prepared using an electrospinning device into nanofiber nonwoven fibers with a thickness of 50 μm in which nanofibers were laminated. As designated in Example 5, BMP was fixed on the

surface of PCL. Subsequently, the nanofiber nonwoven fabrics prepared above were rolled into a 5 mm hole in the DCPA scaffold finally to obtain a DCPA/BMP-fixed PCL nanofiber composite scaffold.

[0059] The composite scaffold thus prepared showed results similar to those obtained in Example 4. It was observed that nanofibers nonwoven fabrics having an average diameter of 300 nm were well combined in the hole in the DCPA ceramic scaffold.

EXAMPLE 9:

[0060] A slurry obtained by mixing HA and bioactive glass powders in a ratio of 90 to 10 was applied on a 60 ppi polyurethane foam and according to the same procedure as in Example 1, a HA/glass ceramic scaffold with a diameter 10 mm and a height of 5 mm, and a hole diameter of 3 mm, was prepared. 500 mg PLGA (50:50, IV: 0.8-1.2) was dissolved in 8 ml dichloromethane and vigorously stirred using an agitator with addition of 10 μm/mL transforming growth factor (TGF) solution. Subsequently, a solution containing a PLGA/TGF mixture was poured into a 0.2 % PVA solution and stirred at room temperature for 4 hours to prepare a TGF mounted PLGA microsphere. The prepared microsphere was then placed into a 3 mm hole in the HA/glass ceramic scaffold finally to obtain a PLGA microsphere composite scaffold containing HA/glass/TGF.

[0061] In the composite scaffold thus prepared, the HA/glass ceramic scaffold achieved results similar to those obtained in Example 4 and the prepared microsphere was found to have an average diameter of 50 μm.

EXAMPLE 10

[0062] A slurry obtained by mixing HA and silica powders in a ratio of 90 to 10 was applied on a 100 ppi polyurethane foam and according to the same procedure as in Example 1, a HA/silica

ceramic scaffold with diameter 20 mm and a height of 10mm, and a hole diameter of 5 mm ,was made. 20 mg vancomycin was added in a 2% gelatin solution until it was completely dissolved. The obtained gelatin/vancomycin solution was left for at least 1 hour at 4□ to be gelled. Subsequently, the gelled gelatin was placed into a 5 mm hole of the HA/silica ceramic scaffold prepared above finally to obtain a gelatin composite scaffold containing HA/silica/vancomycin.

[0063] In the composite scaffold thus prepared, the HA/silica ceramic scaffold achieved results similar to those obtained in Example 5 and it was observed that the gelled gelatin was well incorporated in the hole of the scaffold.

EXAMPLE 11

[0064] A slurry obtained by mixing HA and TTCP powders in a ratio of 75 to 25 was applied on a 80 ppi polyurethane foam and according to the same procedures as in Example 1, an HA/TTCP ceramic scaffold with diameter 20 mm a height of 5 mm, and a hole diameter of 10 mm, was made. The porous collage blocks were prepared using a freeze-drying method and a vascular endothelial growth factor (VEGF) was then fixed on the surface thereof by using EDC and NHS. The collagen scaffold was placed into the 10 mm hole in the prepared HA/TTCP ceramic finally to obtain a HA/TTCP/VEGF-fixed collagen composite scaffold.

[0065] In the composite scaffold thus prepared, the HA/TTCP ceramic scaffold showed results similar to those obtained in Example 4 and it was observed that the collagen in the hole of the scaffold had a porous structure in which pores having a diameter of 20 to 100 μm were interconnected.

EXAMPLE 12

[0066] A slurry obtained from alumina powders was applied on a 60 ppi polyurethane foam and according to the same procedure in Example 1, an alumina porous ceramic scaffold with a

diameter of 10 mm and a height of 5 mm, and having two holes each of which has a diameter of 3 mm, was prepared. The heparin-fixed PLGA film and PLGA nonwoven fabrics containing dexamethasone were prepared using the same procedures as in Examples 4 and 6, respectively, and each was placed into the 5 mm holes in the alumina ceramic scaffold. Finally, an alumina/polymer composite scaffold containing two kinds of bioactive molecules was obtained.

[0067] The composite scaffold thus prepared, as shown in **FIG. 2B**, showed results similar to those obtained in Examples 1, 4, and 6.

EXAMPLE 13

[0068] A slurry obtained by mixing TCP and TTCP powders in a ratio of 80 to 20 was applied on a 100 ppi polyurethane sponge and according to the same procedure as in Example 1, a TCP/TTCP porous ceramic scaffold with a diameter of 15 mm and a height of 5 mm and having three holes each of which has a diameter of 3 mm, was made. The heparin-fixed PLGA, BMP-fixed PCL nanofibers, and TGF-mounted microsphere were prepared using the same procedures as in Examples 4, 8, and 9, respectively, and were each placed into the 3 mm holes in the TCP/TTCP ceramic scaffold finally to obtain a TCP/TTCP/polymer composite scaffold containing three kinds of bioactive molecules.

[0069] The composite scaffold thus prepared showed results similar to those obtained in Examples 4, 8, and 9.

EXAMPLE 14

[0070] A slurry prepared by mixing TCP and DCPA powders in a ratio of 75 to 25 is applied on a 100 ppi polyurethane sponge and according to the same procedure as in Example 1, a TCP/DCPA porous ceramic scaffold with a diameter 20 mm and a height of 10 mm, and having four holes each of which has a diameter of 3 mm, was made. The heparin-fixed PLGA, PLGA

nonwoven fabrics containing dexamethasone, BMP-fixed PCL nanofibers, and TGF-mounted microsphere were prepared using the same procedures as in Examples 4, 6, 8, and 9, respectively, and were each placed into the 3 mm holes in the TCP/DCPA porous ceramic scaffold finally to obtain a TCP/TTCP/polymer composite scaffold containing four kinds of bioactive molecules.

[0071] The composite scaffold thus prepared showed results similar to those obtained in Examples 4, 6, 8, and 9.

EXAMPLE 15

[0072] A slurry obtained by mixing HA and glass powders in a ratio of 80/20 was applied on a 80 ppi polyurethane sponge and according to the same procedure as in Example 1, a HA/glass porous ceramic scaffold with a diameter of 30 mm and a height of 10 mm, and having five holes each of which has a diameter of 3 mm, was made. The heparin-fixed PLGA film, PLGA nonwoven fabrics containing dexamethasone, BMP-fixed PCL nanofibers, TGF-mounted microsphere, and porous collagen containing VGF were prepared using the same procedures as in Examples 4, 6, 8, 9, and 11, respectively, and were each placed into the 3 mm holes in the TCP/DCPA porous ceramic scaffold finally to obtain a HA/glass/polymer composite scaffold containing five kinds of bioactive molecules.

[0073] The composite scaffold thus prepared showed results similar to those obtained in Examples 4, 6, 8, 9, and 11.

EXAMPLE 16

[0074] A slurry obtained by mixing HA, TCP, and glass powders in a ratio of 70:20:10 was covered onto a 100 ppi polyurethane sponge and according to the same procedure as in Example 1, a HA/TCP/glass porous ceramic scaffold with a diameter of 50 mm and a height of 30 mm and having six holes with a diameter of 5 mm, was made. The heparin-fixed PLGA film, PLGA

nonwoven fabrics containing dexamethasone, BMP-fixed PCL nanofibers, TGF-mounted microsphere, gelatin gel containing vancomycin, and porous collagen containing VGF were prepared using the same procedures as in Examples 4, 6, 8, 9, 10, and 11, respectively, and were each placed into the 5 mm holes in the HA/TCP/glass porous ceramic scaffold finally to obtain a HA/TCP/glass/polymer composite scaffold containing six kinds of bioactive molecules.

[0075] The composite scaffold thus prepared showed results similar to those obtained in Examples 4, 6, 8, 9, 10, and 11.

COMPARATIVE EXAMPLE 1:

[0076] In order to compare cell specificity for evaluation, a pure HA porous scaffold containing no bioactive molecules was prepared using the same procedure for preparing the HA porous scaffold in Example 1 as a control group.

EXPERIMENTAL EXAMPLE 1:

[0077] The apatite-coated PLA composite scaffold containing HA/dexamethasone prepared in Example 1 was added in a phosphate-buffered saline (PBS, pH 7.4) and a certain amount of PBS solution was collected for 30 days for analysis of the amount of dexamethasone released from the composite scaffold. The amount was measured at 242 nm using a UV spectrophotometer. In addition, human embryonic palatal mesenchymal (HEPM) cells, the progenitor cells of osteoblasts, were seeded on the composite scaffold prepared in Example 1 and the HA porous scaffold prepared as a control group in Comparative Example 1 for 4 weeks. Their cellular specificity was compared and examined using the amount of proteins and alkaline phosphatase (ALP).

[0078] **FIG. 3A** shows the behavior of dexamethasone released from the composite scaffold in Example 1. It was confirmed therefrom that dexamethasone released from the composite

scaffold prepared by the method of the present invention was stably and continuously released for four weeks of culture. In addition, as illustrated in **FIGS. 3B** and **3C**, after four weeks of culture, larger protein and ALP amounts were observed in the composite scaffold in Example 1 as compared to the control group. It was appreciated therefrom that the PLA composite porous scaffold containing HA/dexamethasone prepared according to the present invention had superior cell specificity. Accordingly, it was confirmed from the above results that the ceramic/polymer composite scaffold containing bioactive molecules according to the present invention can be more effectively used in hard tissue regeneration.

[0079] The present invention is capable of preparing a ceramic scaffold containing bioactive molecules more simply and efficiently than conventional methods. Further, it can utilize the advantages of both ceramics (superior bioactivity) and polymers (biogradable property and ease in processing). In addition, since polymers containing various kinds of bioactive molecules, regardless of form (block, film, fiber, nonwoven fabric, particle, gel, etc.), can be combined to a plurality of ceramic holes, it is possible to prepare a ceramic scaffold containing multiple bioactive molecules, by which it can more effectively induce tissue regeneration when grafted in the body. Bioactive molecules can be directly mixed with a polymer or fixed on a designed surface of a polymer scaffold to be incorporated in the ceramic scaffold. By doing so, the amount and rate of bioactive molecules released therefrom can be controlled. Further, the decomposition of the polymer combined to the ceramic allows for sufficient room for tissues to grow. Therefore, the present invention is expected to solve problems in the prior art that due to their very slow *in vivo* decomposition rate, ceramics hindered the growth of new tissues in a grafted site or caused a foreign body reaction when exposed to the grafted site for a long period. Further, by using a small amount of a biodegradable polymer, the present invention can also solve the problems in prior art that polymers elicite an inflammatory response on a grafted site when decomposed.

What is claimed is:

1. A method of preparing a ceramic/polymer composite scaffold containing bioactive molecules, comprising the steps of:

1) preparing a ceramic scaffold having one or more plurality of holes;

2) dissolving a biodegradable polymer in an organic solvent or aqueous solution and then dissolving or suspending the solution together with bioactive molecules to prepare a polymer solution containing bioactive molecules;

3) placing a polymer into the one or more plurality of holes, wherein said polymer is solidified from the polymer solution containing bioactive molecules inside or outside a hole or said polymer is surface-treated to have bioactive molecules fixed on the surface thereof; and

4) coating the surface of said inorganic/organic composite material with an apatite through biomimic processing.

2. The method according to Claim 1, wherein in step 1), the ceramic is at least one selected from the group consisting of hydroxyapatite (HA), tricalcium phosphate (TCP), tetracalcium phosphate(TTCP), dicalcium phosphate (DCPA), glass ceramics, zirconia, alumina, and composites thereof, and silica/glass- and silica/calcium phosphate based composites.

3. The method according to Claim 1, wherein in step 1), the ceramic scaffold is completely nonporous or has a porosity of 5 to 98%, and has a pore size of 0.1 nm to 5 mm, and the size of the hole in the ceramic scaffold is 0.1 mm to 50 mm and the number of holes ranges from 1 to 10.

4. The method according to Claim 1, wherein in step 1), the hole in the nonporous or porous ceramic scaffold is created in the ceramic scaffold using a drill of 0.5 mm to 50 mm in diameter in a number of 1 to 10, or obtained using a 10 to 100 ppi (pore per inch) of polymer sponge in which a hole of 0.1 mm to 50 mm in diameter has been created in a number of 1 to 10.

5. The method according to Claim 1, wherein in step 1), in preparation of a ceramic slurry, at least one selected from the group consisting of polyvinylalcohol (PVA),

carboxymethylcellulose (CMC), sodium silicate, polyvinyl butyal, methacrylate, water-soluble polyacrylate, polyacrylic acid, polyethylene glycol, ammonium polyacrylate (APA), and N, N-dimethylformamide (DMF) is added in a range of 0.1 to 5% and sintered at a temperature of 600 to 1600°C for one hour.

6. The method according to Claim 1, wherein in step 2), the biodegradable polymer is at least one selected from the group consisting of polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolide) (PLGA), poly-ε-caprolaction (PCL), polyamino acid, polyanhydride, polyorthoester, polyethyleneoxide, fluronic, collagen, gelatin, chitin, chitosan, alginate, hyaluronic acid, fibrinogen, cellulose dextran, pectin, polylysine, albumin, and derivatives and copolymers thereof.

7. The method according to Claim 1, wherein in step 2), the biodegradable polymer has a weight average weight molecular of 500 to 2,000,000 g/mol.

8. The method according to Claim 1, wherein in step 2), the solvent is selected from the group consisting of methylenechloride, chloroform, carbon tetrachloride, acetone, dioxane, tetrahydrofuran, hexafluoroisopropanol, acetic acid, hydrochloric acid, water, and mixtures thereof.

9. The method according to Claim 1, wherein in step 2), the biogradable polymer is added in an amount of 0.01 to 15% by weight relative to the solvent and the bioactive molecules are added in an amount of 10^{-7} to 50% by weight relative to said biogradable polymer.

10. The method according to Claim 1, wherein in step 2), the bioactive molecules are selected from the group consisting of growth factors, growth hormones, peptide medical products, protein medical products, anti-inflammatory drugs, anticancer agents, antiviral agents, sex hormones, antibiotics, antibacterial agents, and compounds.

11. The method according to Claim 10, wherein the bioactive molecules are selected from the group consisting of transforming growth factor (TGF), fibroblast growth factor (FGF), bone

morphogenic protein (BMP), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), placental growth factor (PIGF), granulocyte colony stimulating factor (G-CSF), heparin, heparin, animal growth hormones, human growth hormones (hGH), erythiopoietin (EPO), interferon (INF), follicle stimulating hormone (FSH), progesterone (LH), luteinizing hormone-releasing hormone (LH-RH) agonists of goserelin acetate, leuprolid acetate, and triptoneline acetate, dexamethasone, indomethacin, ibuprofen, ketoprofen, piroxicam, flurbiprofen, diclofenac, etc.; anticancer agents such as paclitaxel, doxorubicin, carboplatin, camptothecine, 5-fluorouracil, ciproretin, cytosine arabinose, methotrexate, etc.; antiviral agents such as acyclovir, testostherone, estrogen, progestheron, estradiol, etc.; antibiotics such as tetracycline, minocycline, doxycycline, ofloxacin, levofloxacin, ciprofloxacin, clarithromycin, erythromycin, cefaclor, cefotaxime, imipenem, penicillin, gentamycin, strentomycin, vancomycin, etc.; antifungal agents such as ketoconazole, itraconazole, fluconazole, amphotericin-B, griseofulvin, etc., and other compounds such as β-glycerophosphate, ascorbate, hydrocortisone, and 5-azacytidine.

12. The method according to Claim 1, wherein in step 3), a polymer block containing bioactive molecules is formed inside or outside a hole in the ceramic scaffold and then cut into said hole, said polymer block having a porosity of 5 to 98% and a pore size of 0.1 nm to 5 mm, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

13. The method according to Claim 1, wherein in step 3), a polymer film containing bioactive molecules is cut or rolled into a hole in the ceramic scaffold, said polymer film being nonporous or having a porosity of 5 to 98%, and a thickness of 0.01 nm to 1 mm, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

14. The method according to Claim 1, wherein in step 3), fiber aggregates containing bioactive molecules are placed into a hole in the ceramic scaffold, said fiber aggregates having a porosity of 5 to 98% and a diameter of 10 nm to 1 mm, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

15. The method according to Claim 1, wherein in step 3), a nonwoven fabric containing bioactive molecules is placed into a hole in the ceramic scaffold, said nonwoven fabric which is composed of fibers each of which has a diameter of 10 nm to 1 mm having a thickness of 10 μm to 20 mm, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

16. The method according to Claim 1, wherein in step 3), polymer particles containing bioactive molecules is placed into a hole in the ceramic scaffold, said polymer particles being nonporous or having a porosity of 5 to 98%, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

17. The method according to Claim 1, wherein in step 3), a polymer gel containing bioactive molecules is placed into a hole in the ceramic scaffold, said polymer gel having a porosity of 5 to 98% and being gelled at a concentration of 0.1 to 90% relative to a solvent and at a temperature of 0 to 90°C, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

18. The method according to Claim 1, wherein in step 3), polymer block, film, fiber, nonwoven fabric, particle, and gel containing bioactive molecules are placed into a hole in the ceramic scaffold in a number of 1 to 10, said polymer block, film, fiber, nonwoven fabric, particle, and gel being nonporous or having a porosity of 5 to 98%, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

19. The method according to Claim 1, wherein step 3), polymer block, film, fiber, nonwoven fabric, particle, and gel are placed into a hole in the ceramic scaffold in a number of 1 to 10, said polymer block, film, fiber, nonwoven fabric, particle, and gel being surface-treated to have bioactive molecules fixed on the surface thereof in around 50% relative to the weight of a polymer used, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

20. The method according to Claim 19, wherein the polymer is surface-treated with a plasma

using a gas selected from the group consisting of argon, oxygen, hydrogen peroxide, and ammonia, or during or after the plasma treatment, hydrophilic polymerization monomers containing functional groups are applied to the surface of the polymer such that bioactive molecules are directly combined to the functional groups or fixed on the surface of the polymer through electric combination.

21. The method according to Claim 20, wherein the hydrophilic polymerization monomers containing functional groups are selected from the group consisting of organic acids containing a carboxy group in the terminal group selected from the group consisting of acrylic acid, maleic acid, itaconic acid, cis-aconic acid, crotonic acid, fumaric acid, trans-flutanic acid, and mixtures thereof; monomers having phosphates in the terminal group selected from the group consisting of vinylphosphonic acid and ethylene glycol methacrylate phosphate (EGMP); and ionic polymers selected from the group consisting of hyaluronic acid, heparine, condroithin sulfate, albumin, polylysine, chitosan, alginic acid, pectin, dextran sulfate, and mixtures thereof.

22. The method according to Claim 1, wherein in step 4), polymer block, film, fiber, nonwoven fabric, particle, and gel containing bioactive molecules are placed into a hole in the ceramic scaffold in a number of 1 to 10, said polymer block, film, fiber, nonwoven fabric, particle, and gel being nonporous or a porosity of 5 to 98%, and being dipped in a simulated body fluid using an alternate dipping process within two days to form an apatite on the surface of the polymer, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

23. The method according to Claim 1, wherein in step 4), polymer block, film, fiber, nonwoven fabric, particle, and gel containing bioactive molecules are placed into a hole in the ceramic scaffold in a number of 1 to 10 and then dipped in a SBF solution using an alternate dipping process within two days to form an apatite on the surfaces of a ceramic and a polymer, said polymer block, film, fiber, nonwoven fabric, particle, and gel being nonporous or having a porosity of 5 to 98%, said ceramic scaffold with 1 to 10 holes each of which has a diameter of 0.1 mm to 50 mm.

24. The method according to Claim 1, wherein in step 4), an apatite is formed using a SBF solution on the surface of a polymer or a ceramic and a polymer, said SBF solution being used at a one- to five fold ionic concentration.

25. Ceramic/polymer composite scaffolds containing bioactive molecules prepared by the method of Claim 1.

FIG. 1A

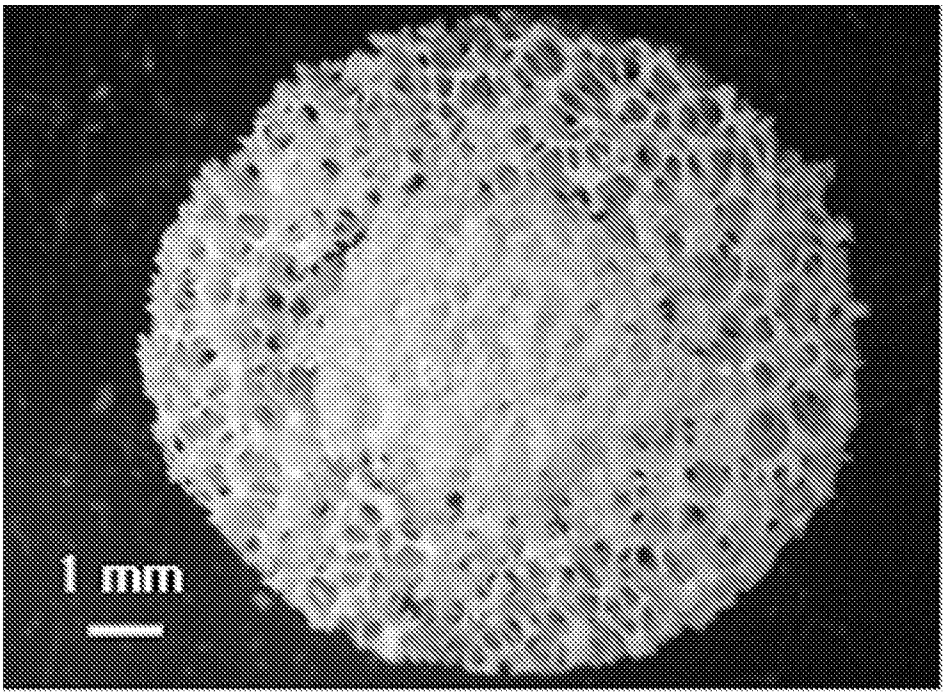


FIG. 1B

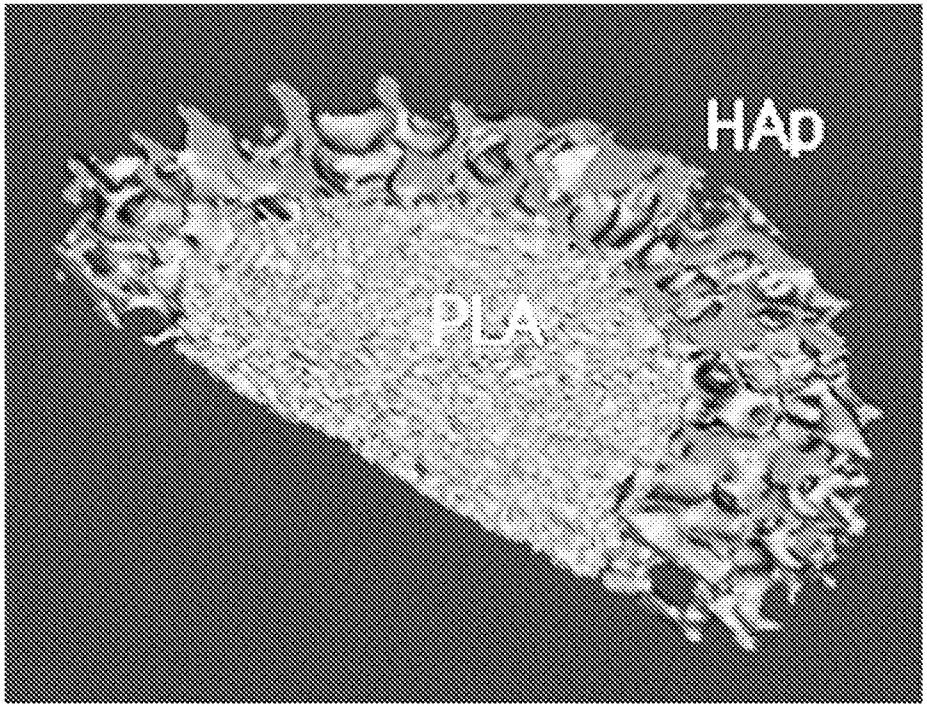


FIG. 1C

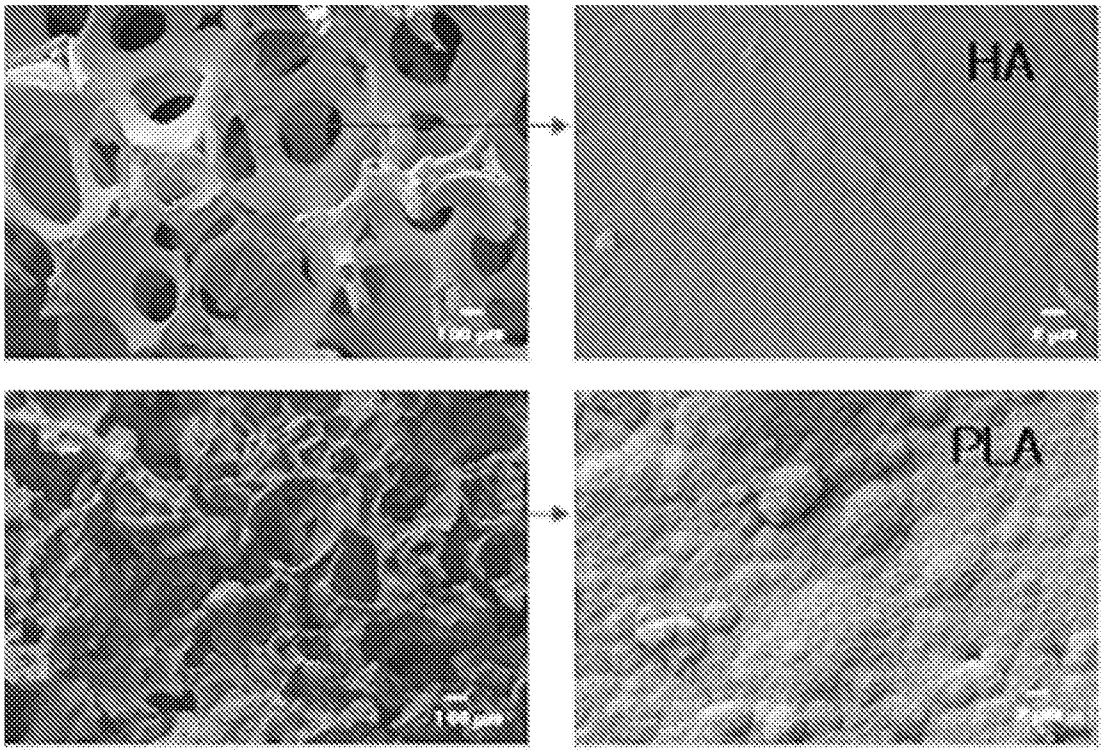


FIG. 2A

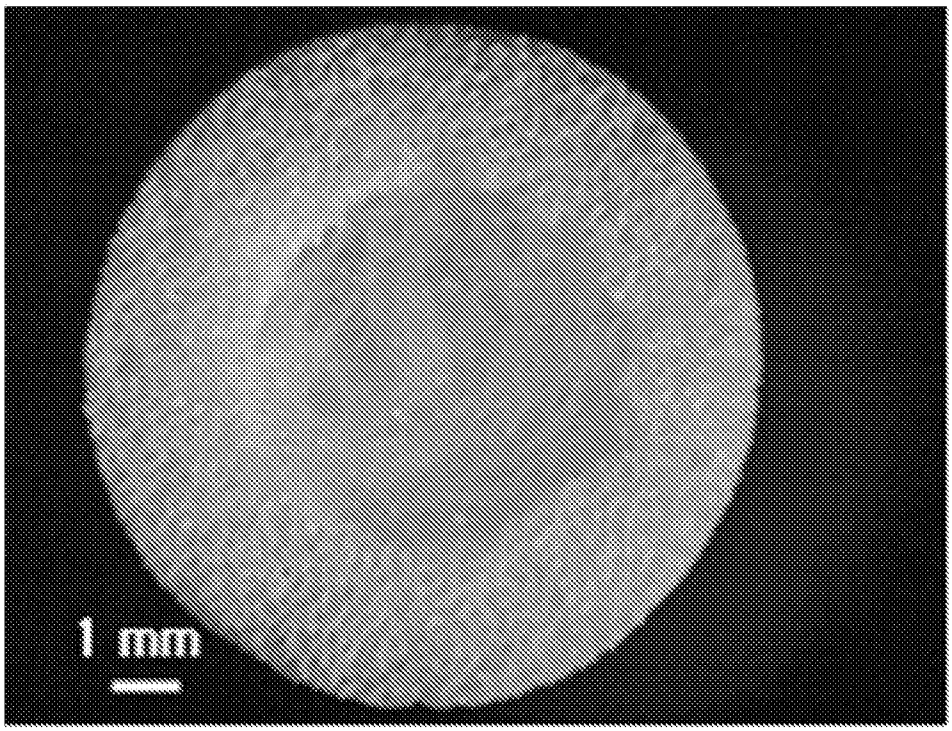


FIG. 2B

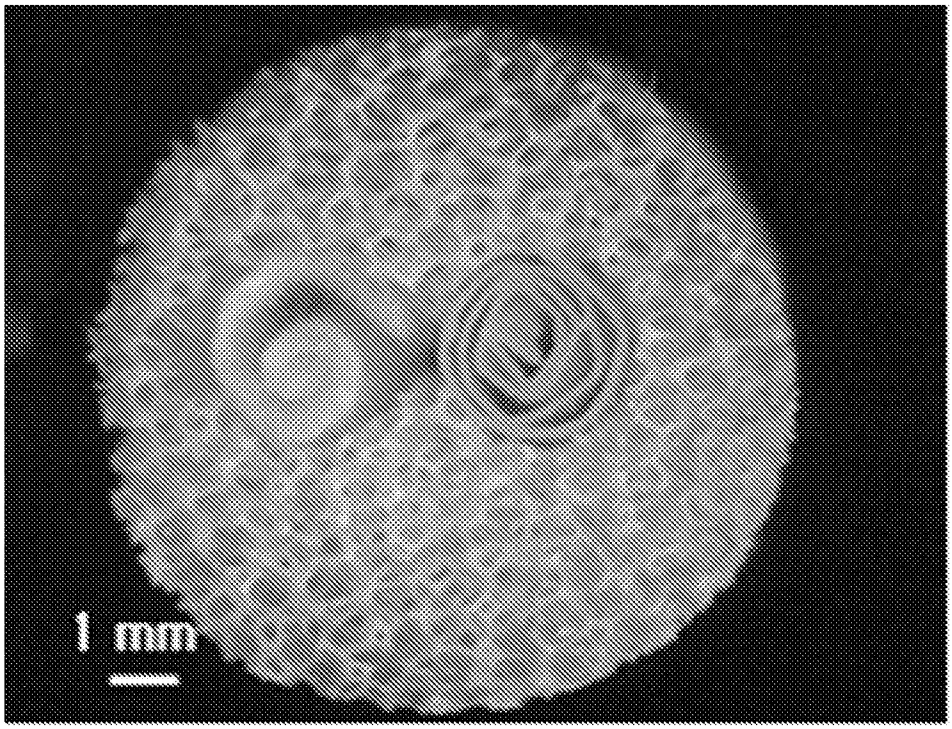


FIG. 3A

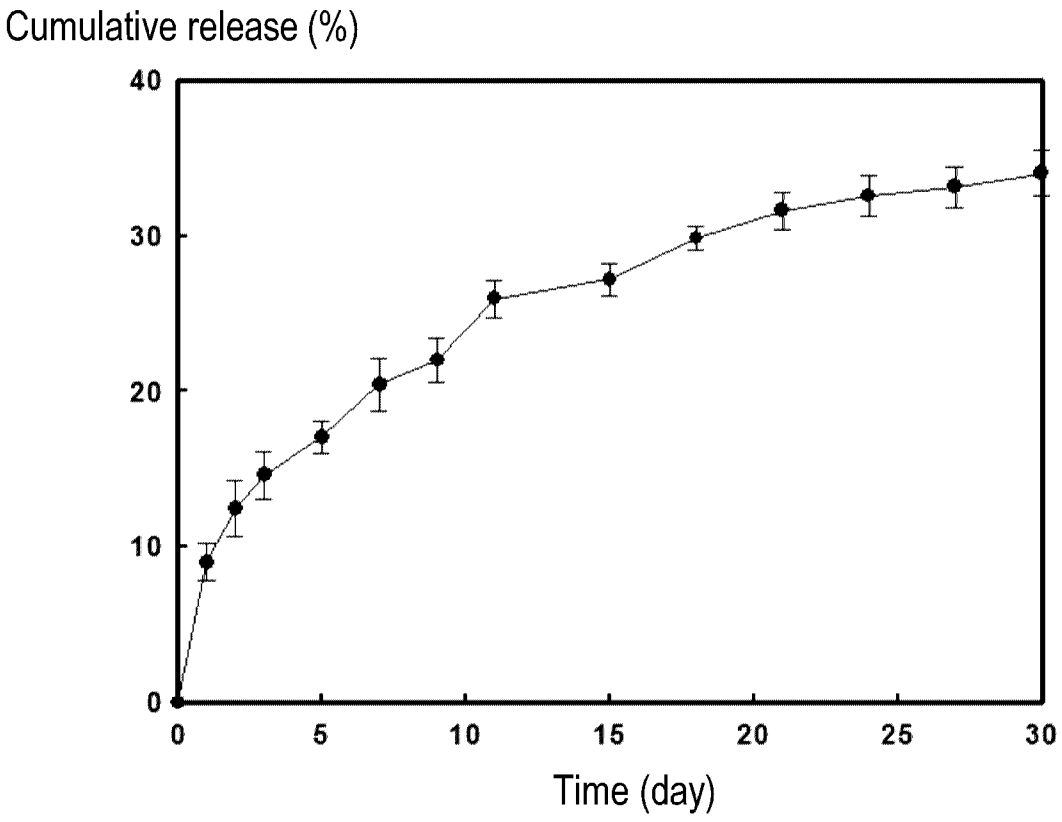


FIG. 3B

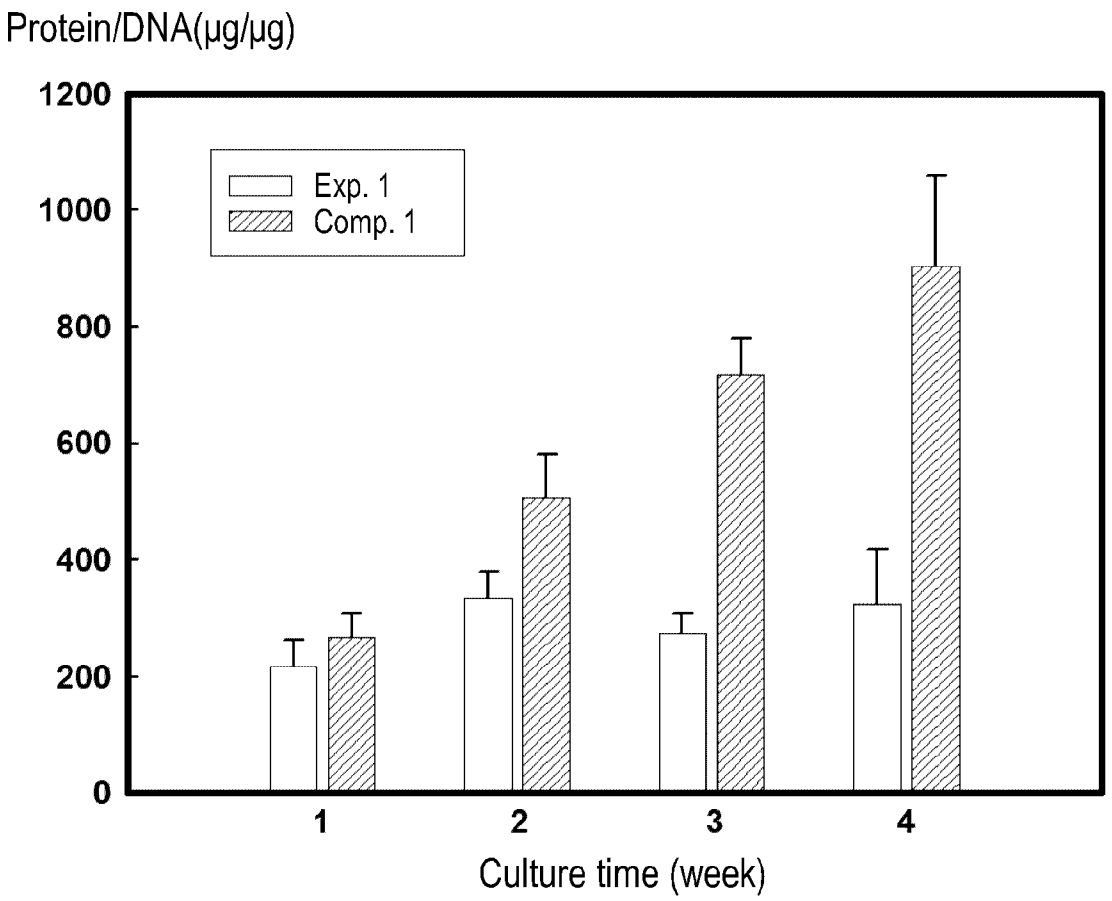
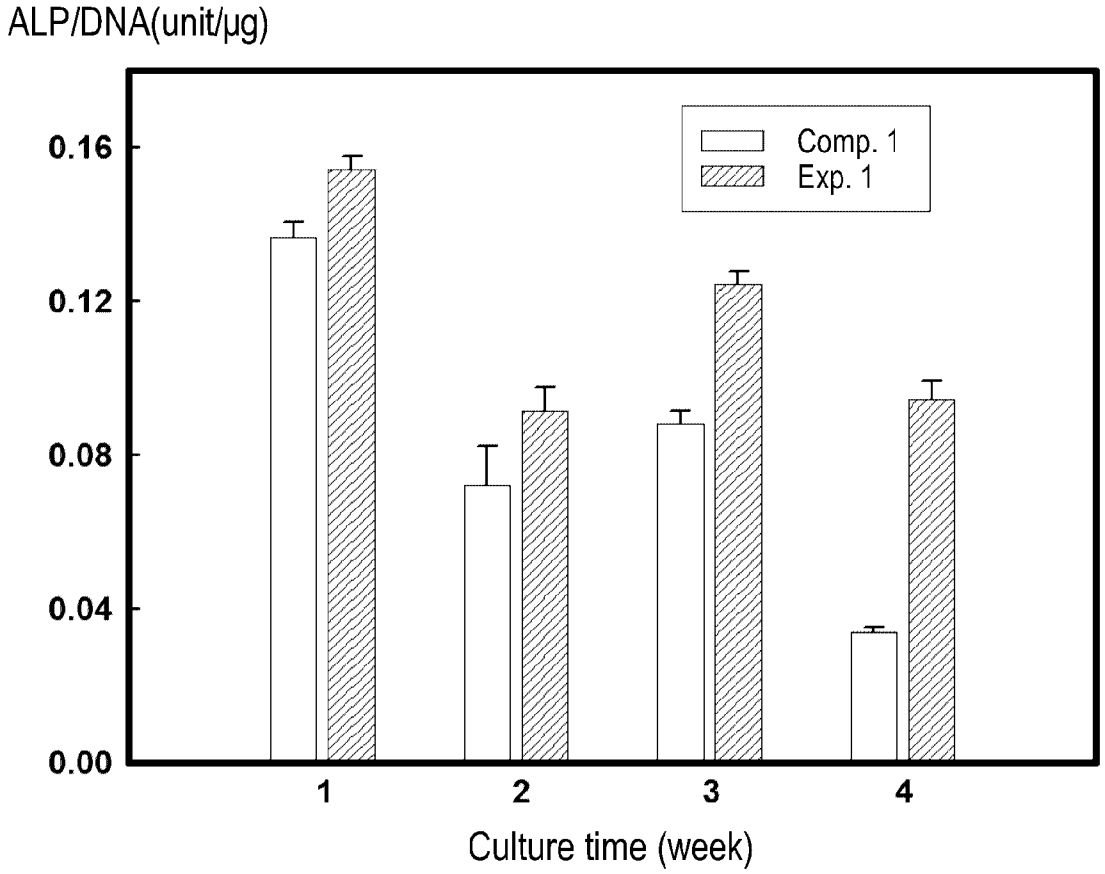

Protein/DNA(μg/μg)
1200
1000
800
600
400
200
0
Exp. 1
Comp. 1
1
2
3
4
Culture time (week)

FIG. 3C


ALP/DNA(unit/μg)
0.16
0.12
0.08
0.04
0.00
Comp. 1
Exp. 1
1
2
3
4
Culture time (week)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/029396

A. CLASSIFICATION OF SUBJECT MATTER

A61L 27/10(2006.01)i, A61L 27/56(2006.01)i, A61L 27/14(2006.01)i, A61L 27/54(2006.01)i, A61L 27/12(2006.01)i, A61F 2/02(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)

A61L 27/10; A61P 43/00; A61L 27/44; A61K 9/00; C01B 25/10; A61K 6/083; A61L 27/56; A61B 17/08; A61L 33/00

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: porous(holes), ceramic, biodegradable polymer, apatite, solvent, growth factors

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1604696 A1 (ETHICON, INC.) 14 December 2005 See abstract; paragraphs 0001,0012,0028,0040,0048; claims 1,10,12-14,21.	1-25
A	US 2010-0010513 A1 (YUN HUISUK et al.) 14 January 2010 See abstract; paragraphs 0012-0015,0017,0033-0035,0037,0057,0064; claims 1,3-5,7,18,20.	1-25
A	US 2010-0047318 A1 (KUMAR MUKESH) 25 February 2010 See abstract; paragraphs 0004,0013,0013-0018,0020,0022,0032,0033; claims 1,7-10,14.	1-25
A	US 2006-0198939 A1 (TIMOTHY SMITH et al.) 07 September 2006 See abstract; paragraphs 0001,0031,0034,0067,0088,0089; claims 1,5,6,8,11,44,46.	1-25
A	US 2006-0199876 A1 (TROCZYNSKI et al.) 07 September 2006 See abstract; claims 1,28,35.	1-25

☐ 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

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"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
30 MAY 2011 (30.05.2011)	**30 MAY 2011 (30.05.2011)**

Name and mailing address of the ISA/KR

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Telephone No. 82-42-481-8655

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2010/029396

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US 2006-0199876 A1	07.09.2006	None	