

CAVELIER

ABOGADOS

www.cavelier.com
cavelier@cavelier.com

EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTÁ 8, CO

297
298
Teléfono: (57-1) 347 3611
Telefax: (57-1) 211 8650
(57-1) 540 0860
(1-305) 381 9660

Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-092514- -00004-0000

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Evento : 378
Actuación : 538

Referencia : Expediente No. 07.092.514
Asunto : Solicitud de Patente para **UN BIOMATERIAL COMPUESTO QUE COMPRENDE UN MATERIAL INORGANICO Y UN MATERIAL ORGANICO Y SU PROCESO DE PREPARACION**
Solicitante : **CAMBRIDGE ENTERPRISE LIMITED & MASSACHUSETTS INSTITUTE OF TECHNOLOGY**
Clase : A61L 027/046, A61L 027/056
Publicada en : La Gaceta No. 590 del 31 de marzo de 2008
Fecha de Solicitud : 7 de septiembre de 2007

1. Yo, Emilio FERRERO, identificado como aparece al pie de mi firma, actuando como apoderado en el asunto de la referencia, atentamente, solicito se efectúe el examen de patentabilidad de la solicitud en referencia presentada de acuerdo con el Capítulo I del PCT y según lo establecido en el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina. Para tal efecto presento junto con este escrito el recibo por valor de \$ 420.000.00, que corresponde a la tasa fijada según lo dispuesto en la resolución No. 44372 de 26 de diciembre de 2007.

2. Ruego atentamente a la Señora Jefe se sirva ordenar que se anexe este recibo para que se efectúe dicho examen y se continúe con el trámite de la solicitud.

Señora Jefe,

EMILIO FERRERO
T.P.A. No. 31.929

COLO-16112-841-001-6
SDC\NNJ\ID-132040\WPC16112
No. M-2008-132040\SDC

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-92514

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **590** DE

FECHA **31 DE MARZO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **27 DE JUNIO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO X

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

Bogotá, D.C., 04 de marzo de 2011

Apoderado: Emilio Ferrero

ASUNTO Radicación 07-92514
 Trámite 002
 Evento 001
 Actuación 430
 Oficio
 Folios

05103

Patente de Invención ☒

Vía PCT fase nacional



Cap. I



Cap. II



No. Sol. Internacional

PCT/EP2005/055753

Fecha de Presentación Internal. 04/nov/2005

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

1.1. Descripción: Folios: 123 a 217 como se radicó inicialmente

1.2. Reivindicaciones: Folios 218 a 229 Como se radicó inicialmente

1.4 Prioridad: Folio 2: GB 0504673.5; 07/mar/2005

1. Objeto de la invención

§ Título de la solicitud: BIOMATERIAL

§ El objeto de la invención se refiere a un proceso para la obtención la obtención de un biomaterial de hueso sintético y el biomaterial sintético.

-Reivindicaciones 1-32: Un proceso para la preparación de un biomaterial.

-Reivindicaciones 33-34, 46-52: Un biomaterial.

-Reivindicaciones 35-36: Un biomaterial.

-Reivindicaciones 37-38: Un biomaterial.

-Reivindicaciones 43-45: Un biomaterial.

-Reivindicación 55: Un biomaterial, obtenido por el proceso de las reivindicaciones 1 a 32.

§ El problema planteado en esta solicitud es la necesidad de un material sintético que tenga una composición y estructura que permita el crecimiento del hueso natural.

§ Para solucionar este problema técnico la solicitud en estudio proporciona un proceso para la preparación de un biomaterial.

Se catalogó la invención, según la Clasificación Internacional de Patentes (IPC.): A61L27/46; A61L27/56

2. De la solicitud de Patente

2.1. Reivindicaciones (artículo 30 D 486 de la C.A.N.)

-Las reivindicaciones 40-34 son inconsistentes, hacen referencia a una segunda capa de acuerdo con las reivindicaciones 33-36 y esas reivindicaciones no menciona ninguna segunda capa, de igual manera quedan con la misma inconsistencia todas las que son dependientes de estas.

-Las reivindicaciones 53 y 54, no son claras, ya que no definen "*el material óseo sintético, implante óseo, sustituto óseo, soporte óseo, relleno, recubrimiento o cemento, como tampoco se define el soporte óseo monolítico*".

Las expresiones funcionales no comprenden información alguna con respecto a la conformación inequívoca de la materia reclamada en estas reivindicaciones, o sea, es un resultado por obtener.

Por lo tanto las reivindicaciones no cumplen con el requisito de claridad, concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486.

3. **Unidad de invención** (artículo 25 D 486)

-En el capítulo reivindicatorio se no conserva la unidad inventiva ya que cada uno de los grupos no comprende las características técnicas especiales de la invención, características técnicas especiales son aquellas características que proporcionan un aporte técnico real al acervo tecnológico:

- i) Un proceso para la preparación de un biomaterial; un biomaterial, obtenido por el proceso de las reivindicaciones 1 a 32.
- ii) Un biomaterial (reivindicaciones 33-34,46-52).
- iii) Un biomaterial (reivindicaciones 35-36).
- iv) Un biomaterial (reivindicaciones 37-38).
- v) Un biomaterial (reivindicaciones 43-45)
- vi) Reivindicaciones 53 Material óseo sintético.

4. Determinación del Estado de la Técnica

Considerando la fecha de prioridad reclamada de la presente solicitud de patente 07/mar/2005, se realizó la búsqueda del estado de la técnica, en bases de datos donde ésta oficina tiene acceso libre, para búsquedas de documentos relacionados con la materia que se reclama como nueva e inventiva en la presente solicitud.

Encontrándose los siguientes documentos relevantes

Nº	Documento	Título	Fecha de Publicación*
D1	WO03092759	Method for preparing porous composite material	13/nov/2003
D2	WO03092760	Structured composites as a matrix (scaffold) for the tissue engineering of bones	13/nov/2003
D3	WO2004041320	Apattite/collagen crosslinked porous material containing self-organized apattite/colagen composite and process for producing the same	21/may/2004
D4	US5320844	Composite materials for hard tissue replacement	14/jun/1994

5. Análisis de patentabilidad (según requisito) (artículo 14 D486)

5.1. Novedad (artículo 16 D486)

La solicitud en estudio reclama *un proceso para la preparación de un biomaterial compuesto que comprende un material inorgánico y un material orgánico, el proceso comprende:*

- Suministrar una primera composición de suspensión que comprende un portador líquido, un material inorgánico y un material orgánico;*
- Suministrar un molde para la suspensión;*
- Depositar la suspensión;*
- Enfriar la suspensión depositada en el molde a una temperatura en la que el portador líquido se transforma en una pluralidad de partículas o cristales sólidos mediante sublimación y/o evaporación para dejar un material compuesto poroso que comprende un material inorgánico y un material orgánico; y*
- Remover al menos algo de la pluralidad de las partículas o cristales sólidos mediante sublimación y/o evaporación para dejar un material compuesto poroso que comprende un material inorgánico y un material orgánico; y*
- Remover material del molde.*

-La anterioridad D1 enseña un procedimiento para producir un material poroso y a los materiales porosos. El procedimiento comprende los pasos de proporcionar

cuales es gelatinizada, al resultante se le sublima el portador (lío­filizado). El material poroso y los materiales obtenidos a partir de este, elaborados por el método de la presente invención con las fuerzas mecánicas adecuadas pueden tener diámetros poro grande, altas porosidades, y buena biodegradabilidad. Por lo tanto son adecuado para implantes óseos, por ejemplo los materiales de carga del hueso, vehículos de fármacos para de liberación sostenida y similares.

Véase la anterioridad especialmente

-La anterioridad D2 describe un material compuesto, estructurado como vehículo para la ingeniería del tejidos y el material de implante óseo, el cual consiste en una composición del fosfato cálcico poroso que granula desde la composición que lo contiene. El material granulado tiene tamaños de grano entre de 0.5 y 10 mm con macroporos y de microporos; los macroporos tienen un diámetro medio entre del 50-500 µm y están unidos por un agente ligante biocompatible.

Véase la anterioridad especialmente pág. 1, renglones 4-9, el ejemplo, reivindicaciones 1,2,4,7,8,12,13.

-D3 enseña un procedimiento para producir un material poroso entrecruzado que comprende un material inorgánico y un material orgánico (apatita/colágeno), comprendiendo gelificándose una dispersión que contiene un material compuesto por apatita/colágeno y el colágeno, luego liofilización del gel de tal modo que se forma un cuerpo poroso y reticulación del colágeno del cuerpo poroso y el material poroso entrecruzado de apatita/colágeno producido por este procedimiento.

Véase la anterioridad especialmente los párrafos [0011] a [0025], junto con las reivindicaciones 1, 2,11,12.

-La anterioridad D4 describe un material compuesto sintético, substancialmente homogéneo que contiene un componente de colágeno y los componentes con fosfato y calcio son precipitados de un medio líquido en el cual se contenga el componente de colágeno.

Véase la anterioridad, especialmente colum.2 renglón 16 - colum.3 renglón13; colum.3 renglones 35 – 54; colum.3 renglón 62 – colum.5 renglón 31; ejemplos 1 y 2; reivindicaciones.

Tal como se puede ver la materia que se reclama como nueva e inventiva en la presente solicitud está comprendida en las anterioridades D1-D4 por lo tanto no se observa un aporte que enriquezca al acervo tecnológico, diferente al aporte ya *hecho* por las anterioridades citadas

Si existen oposiciones por parte de terceros, demostrar cómo los fundamentos de éstas afectan la Novedad es decir cómo los objetos se consideran idénticos.

6. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

**Una solicitud que no posee novedad obviamente no ostenta nivel inventivo.*

D1	WO03092759	1-6,20-23,25,28-30,33-36,47-51,53-55	Novedad
D2	WO03092760	1-7,20-23,25,28-30,33-36,47-51,53-55	Novedad
D3	WO2004041320	1-55	Novedad
D4	US5320844	1-6,20-23,25,28-30,33-36,47-51,53-55	

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el artículo 8 de la resolución reglamentaria No. 00210 del 15 de enero de 2001, advirtiéndole que contra él no procede recurso alguno en la vía gubernativa.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha

18 MAR 2011

CONCEPTO TECNICO No.	EXAMINADOR TECNICO Cód N°202012
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国際事務局(43) 国際公開日
2003 年 11 月 13 日 (13.11.2003)

PCT

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(22) 国際出願日: 2002 年 5 月 1 日 (01.05.2002)

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(26) 国際公開の言語: 日本語

(71) 出願人 (米国を除く全ての指定国について): 科学技術振興事業団 (JAPAN SCIENCE AND TECHNOLOGY CORPORATION) [JP/JP]; 〒332-0012 埼玉県 川口市 本町四丁目 1 番 8 号 Saitama (JP). 独立行政法人物質・材料研究機構 (NATIONAL INSTITUTE FOR MATERIALS SCIENCE) [JP/JP]; 〒305-0047 茨城県 つくば市千現一丁目 2 番 1 号 Ibaraki (JP). 独立行政法人産業技術総合研究所 (NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY) [JP/JP]; 〒100-8921 東京都 千代田区霞が関一丁目 3 番 1 号 Tokyo (JP).

(72) 発明者; および

(75) 発明者/出願人 (米国についてのみ): 早乙女 進一 (SOTOME, Shinichi) [JP/JP]; 〒108-0023 東京都 港区芝浦 4-18-30 フロンティア芝浦 710 Tokyo (JP). 植村 寿公 (UEMURA, Toshimasa) [JP/JP]; 〒

305-0044 茨城県 つくば市並木 3-674-101 Ibaraki (JP). 田中 順三 (TANAKA, Junzo) [JP/JP]; 〒305-3254 茨城県 つくば市鹿島台 3-6 Ibaraki (JP). 菊池 正紀 (KIKUCHI, Masanori) [JP/JP]; 〒305-0032 茨城県 つくば市竹園 3-15-109-202 Ibaraki (JP). 四宮 謙一 (SHINOMIYA, Kenichi) [JP/JP]; 〒114-0014 東京都 北区田端 5-12-13 パインマンション 502 Tokyo (JP). 伊藤 聡一郎 (ITO, Soichiro) [JP/JP]; 〒112-0005 東京都 文京区水道 1-11-7 シャンボール小石川 901 Tokyo (JP). 立石 哲也 (TATEISHI, Tetsuya) [JP/JP]; 〒305-0042 茨城県 つくば市下広岡 670-3 Ibaraki (JP).

(74) 代理人: 平木 祐輔, 外 (HIRAKI, Yusuke et al.); 〒105-0001 東京都 港区 虎ノ門一丁目 17 番 1 号 虎ノ門 5 森ビル 3 階 Tokyo (JP).

(81) 指定国 (国内): JP, US.

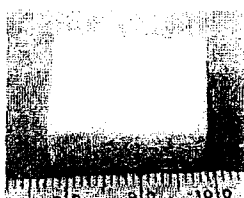
(84) 指定国 (広域): ヨーロッパ特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

添付公開書類:
— 国際調査報告書

2 文字コード及び他の略語については、定期発行される各 PCT ガゼットの巻頭に掲載されている「コードと略語のガイダンスノート」を参照。

(54) Title: METHOD FOR PREPARING POROUS COMPOSITE MATERIAL

(54) 発明の名称: 多孔質複合材料の製造方法



A: 45°C 30min

B: 45°C 90min

(57) Abstract: A method for preparing a porous composite material, which comprises the steps of freezing a composite comprising one or more calcium salts selected from among calcium carbonate, calcium phosphate and hydroxyapatite, and a collagen at least a part of which has been gelatinized, and then subjecting the frozen composite to a lyophilization. A porous composite material prepared by the method has a large pore diameter and a high porosity, as well as appropriate mechanical strength characteristics and biodegradability, and thus can be suitably used as an implant such as a filler for a bone, a carrier for use in the sustained release of a drug, and the like.



(11) EP 1 500 405 A1

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and Technology Agency
t, Saitama 332-0012 (JP)
ute for Materials Science
baraki 305-0047 (JP)
ute of Advanced Industrial
echnology
21 (JP)

nichi
yo 108-0023 (JP)

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- UEMURA, Toshimasa
Tsukuba-shi, Ibaraki 305-0044 (JP)
- TANAKA, Junzo
Tsukuba-shi, Ibaraki 305-3254 (JP)
- KIKUCHI, Masanori
Tsukuba-shi, Ibaraki 305-0032 (JP)
- SHINOMIYA, Kenichi
Kita-ku, Tokyo 114-0014 (JP)
- ITOH, Soichiro
Bunkyo-ku, Tokyo 112-0005 (JP)
- TATEISHI, Tetsuya
Tsukuba-shi, Ibaraki 305-0042 (JP)

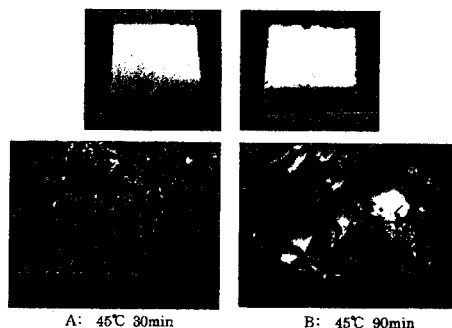
(74) Representative: VOSSIUS & PARTNER
Siebertstrasse 4
81675 München (DE)

FOR PREPARING POROUS COMPOSITE MATERIAL

n relates to a process for produc-
posite materials comprising steps
x containing at least one calcium
calcium carbonate, calcium phos-
phate and collagen, at least a part
d; and then lyophilizing the result-

ant. The porous and composite materials obtained by
the method of the present invention have large pore di-
ameters, high porosities, and adequate mechanical
strengths and biodegradability. Thus, they are suitable
for implants such as bone fillers, drug carriers for sus-
tained-release, and the like.

Fig.2



A: 45°C 30min

B: 45°C 90min

Description

Technical Field

[0001] The present invention relates to a process for producing porous and composite materials. More particularly, the present invention relates to a process for producing porous and composite materials having large pore diameters, high porosities, and mechanical strengths that are suitable for implants such as bone fillers.

Background Art

[0002] In the past, bone defects used to be restored by a technique of transplanting a part of a subject's own bones or a technique of complementing or replacing bones with artificial implants. Such alternative bone implants were required to have properties such as bioadaptability or bone conductivity and bone inductivity (a property of incorporating bone tissue and accelerating bone formation) in addition to mechanical properties similar to those of biological bones. Accordingly, porous materials such as porous ceramics used to be preferably employed as alternative bone implants because they were easily penetrated by bone tissues and they had good bone conductivity and bone inductivity.

[0003] Currently commercialized porous hydroxyapatite, however, is nonabsorbable. Even though this hydroxyapatite was porous, cells could not penetrate pores that did not communicate with the exterior because of this nonabsorbability. Accordingly, the final strength was not very high. Also, it was likely to collapse upon moisture absorption during surgical operations. Thus, the use thereof in surgical operation, which required strength, was difficult.

[0004] Bone fillers of porous β -TCP, which is bioabsorbable and has porosity of 75%, are commercialized (Osferion®, Olympus Optical Co., Ltd.; average pore diameters: 100 μ m to 400 μ m). This β -TCP is highly likely to collapse when it is handled. Thus, it was difficult to prepare this material in a form that is adequate for the transplant site, and was difficult to handle since implants easily become detached from the transplant site.

[0005] Under the above circumstances, the present inventors have conducted various studies to develop a composite material of hydroxyapatite and collagen that has a structure similar to that of biological bones and to improve its properties. For example, JP Patent Publication (Kokai) No. 7-101708 A (1995) discloses a process for producing a complex of apatite and an organic substance having the Young's modulus similar to that of biological bones with the gradual addition of a mixed solution of collagen and phosphoric acid in a suspension of calcium hydroxide. JP Patent Publication (Kokai) No. 11-199209 A (1999) discloses a complex that has a structure similar to that of biological bones can be realized by regulating pH and temperature at the time of re-

action. Further, JP Patent Publication (Kokai) No. 2000-5298 A discloses a technique for enhancing the formation of apatite on a collagen surface with the use of organic acid.

[0006] In spite of these efforts, however, many complexes of hydroxyapatite and collagen that were obtained by the conventional technique had small pore diameters (approximately 0 μ m to 100 μ m) and had low porosities (50% or lower).

[0007] An object of the present invention is to provide novel porous and composite materials having large pore diameters, high porosities, and mechanical strengths that are suitable for implants such as bone fillers.

[0008] The present inventors have conducted concentrated studies in order to attain the above object. As a result, they have found that composite materials having large pore diameters and high porosities could be obtained by gelatinizing a part of the collagen constituting a complex of hydroxyapatite and collagen, followed by freezing and lyophilization thereof. They have also found that application of surface crosslinking to the composite material realized mechanical strengths that were suitable for implants such as bone fillers. This has led to the completion of the present invention.

[0009] More specifically, the present invention provides the following (1) to (10).

(1) A process for producing porous and composite materials comprising steps of: freezing a complex containing at least one calcium salt selected from calcium carbonate, calcium phosphate, and hydroxyapatite and collagen, at least a part of which is gelatinized; and then lyophilizing the resultant.

(2) A process for producing porous and composite materials comprising a step of introducing surface crosslinking between collagens to the porous and composite materials obtained by the process according to (1) above.

(3) The process according to (2) above, wherein the step of introducing surface crosslinking is carried out by immersing the porous and composite materials in a solution containing a crosslinking agent.

(4) The process according to any one of (1) to (3) above comprising a step of adding a crosslinking agent to gelatinized collagen to introduce internal crosslinking between collagens.

(5) The process according to any one of (1) to (4) above, wherein the calcium salt is hydroxyapatite.

(6) A process for producing porous and composite materials comprising the following steps of:

1) gelatinizing at least a part of the collagen constituting a complex containing hydroxyapatite and collagen;

2) introducing internal crosslinking between collagens with the addition of a crosslinking agent to the complex;

3) obtaining porous and composite materials by

complex and then lyophilizing the
d
ing surface crosslinking between
y immersing the porous and com-
materials in a solution containing a
agent.

according to any one of (2) to (6)
internal and/or surface crosslinking
is introduced using glutaralde-
hyde crosslinking agent.

according to any one of (1) to (7)
the resulting porous and composite
porosities of 80% or higher.

composite materials having porosi-
ties higher, which are produced by the
g to any one of (1) to (8) above.
the porous and composite materi-
(9) above.

ention

ucing porous and composite

invention relates to a process for
d composite materials of at least
cted from calcium phosphate, cal-
hydroxyapatite and collagen.

duction process according to the
posite materials having a large
ous and composite materials) are
a complex containing at least one
from calcium carbonate, calcium
xyapatite and collagen, at least a
nized; and then lyophilizing the re-
linking between collagens is intro-
porous and composite materials.
s and composite materials to have
that are suitable for implants.
tion process according to the
hereafter described in detail.

ning calcium salt and collagen, at
s gelatinized

and composite materials accord-
ention are produced from a com-
calcium salt selected from calcium
carbonate, and hydroxyapatite and
art of which is gelatinized.

xyapatite)

salt" that constitutes the complex
on is at least one selected from
calcium carbonate, and hydroxya-
droxyapatite is the most prefera-

ble.

[0015] Hydroxyapatite is generally denoted by a
chemical formula of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, and it includes a
group of compounds referred to as calcium phosphate
such as CaHPO_4 , $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_4\text{O}(\text{PO}_4)_2$, $\text{Ca}_{10}(\text{PO}_4)_6$
(OH)₂, $\text{CaP}_4\text{O}_{11}$, $\text{Ca}(\text{PO}_3)_2$, $\text{Ca}_2\text{P}_2\text{O}_7$, and Ca
(H_2PO_4)₂· H_2O . Also, hydroxyapatite is basically com-
posed of a compound represented by formula Ca_5
(PO_4)₃OH or $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and a part of the Ca
component may be substituted with at least one mem-
ber selected from Sr, Ba, Mg, Fe, Al, Y, La, Na, K, H,
and the like. A part of the (PO_4) component may be sub-
stituted with at least one member selected from VO_4 ,
 BO_3 , SO_4 , CO_3 , SiO_4 , and the like. A part of the (OH)
component may be substituted with at least one mem-
ber selected from F, Cl, O, CO_3 , and the like. Some of
these components may be deficient. In general, a part
of apatite PO_4 and OH components in biological bones
are substituted with CO_3 . Accordingly, the aforemen-
tioned components may be partially substituted by in-
clusion of CO_3 in the air (about 0% to 10% by mass)
during the production of the porous and composite ma-
terials of the present invention.

[0016] Hydroxyapatite is generally microcrystalline,
noncrystalline, or crystalline. Alternatively, it may exist
in the form of an isomorphous solid solution, substitutional
solid solution, or interstitial solid solution. The atomic ra-
tio of calcium/phosphorus (Ca/P) in this "hydroxyapa-
tite" is preferably in the range between 1.3 and 1.8. In
particular, the range between 1.5 and 1.7 is more pref-
erable. When the atomic ratio is in the range between
1.3 and 1.8, the composition and the crystal structure of
apatite in the product (a calcium phosphate compound)
can be similar to those in bones of vertebrates. This im-
proves biocompatibility and bioabsorbability.

2) Collagen

[0017] The "collagen" that constitutes the complex of
the present invention includes a general collagen mole-
cule that has the triple helix structure and gelatinized
"denatured collagen," the triple helix structure of which
is destroyed by thermal or other treatment.

[0018] At present, about 20 different molecular spe-
cies of collagens are known to be present in a wide va-
riety of animal tissues ranging from mammals to fish.
These are generically denoted as "collagens." The spe-
cies, the location of tissue, the age, and other factors
regarding the animal that is a starting material for the
collagen used in the present invention are not particu-
larly limited, and any type of collagen can be used. In
general, collagens obtained from skin, bones, cartilage,
tendons, organs, or the like of mammals (such as
cow, pig, horse, rabbit, or mouse) and birds (such as
chicken) are used. Also, collagen-like proteins obtained
from skin, bones, cartilage, fins, scales, organs, or the
like of fish (such as cod, left-eyed flounder, right-eyed
flounder, salmon, trout, tuna, mackerel, sea bream, sar-

dine, or shark) may be used as starting materials. Alter-
natively, collagen may be obtained by gene recombina-
tion techniques instead of by extraction from animal tis-
sues.

[0019] Among the molecular species of collagens, the
quantity of type I collagens is the largest, and they have
been well studied. In general, when simple reference is
made to a "collagen," it often indicates type I collagen.
The molecular species of the collagen used in the
present invention is not particularly limited, and type I
collagen is preferably a main component. Further, col-
lagen may be prepared by adequately subjecting an
amino acid residue of the collagen protein to chemical
modification such as acetylation, succination, maleyla-
tion, phthalation, benzoylation, esterification, amida-
tion, or guanidination.

[0020] An example of a process for preparing colla-
gen is extraction from the aforementioned starting ma-
terial (excluding the gene recombination technique) with
the aid of a neutral buffer or dilute acid such as hydro-
chloric acid, acetic acid, or citric acid. Collagen obtained
with the aid of the former is referred to as neutral salt-
soluble collagen, and that obtained with the aid of the
latter is referred to as acid-soluble collagen. However,
the amount of collagen extracted is small in either case,
and a majority thereof remains as insoluble collagen.
Enzyme solubilization and alkali solubilization are
known as methods for solubilizing this insoluble colla-
gen. Collagen obtained by the former method is referred
to as enzyme-solubilized collagen, and that obtained by
the latter method is referred to as alkali-solubilized
collagen. Both can be solubilized as molecular collagens
with yields of substantially 100%.

[0021] The method for preparing collagen used in the
present invention (extraction type) is not particularly lim-
ited. If the molecular weight of solubilized collagen is
large, however, the strength of a complex becomes in-
sufficient because of steric hindrance. Accordingly, the
use of monomeric (monomolecular) collagen is prefer-
able. In enzyme-solubilized collagen and alkali-solubi-
lized collagen, the monomeric collagen content is high,
and non-helical regions (telopeptides) having a majority
of collagen antigenicity are selectively degraded and re-
moved during the step of production. Thus, they are par-
ticularly adequate for the organic or inorganic porous
and composite materials of the present invention. If
these non-helical regions are degraded and removed
from collagen, this collagen is referred to as "atelocol-
lagen."

[0022] The isoionic point of enzyme-solubilized colla-
gen is different from that of alkali-solubilized collagen.
The isoionic point is the pH level where both positive
and negative charges, which are derived from a disso-
ciable group inherent to a protein molecule, repel each
other. In the case of collagen, when the pH level ap-
proaches the region of the isoionic point, solubilized col-
lagen is known to become fibrous. In general, the iso-
ionic point of the enzyme-solubilized collagen is between

pH 8 and 9, and that of the alkali-solubilized collagen is
between pH 4 and 5. In the present invention, it is pref-
erable to use the enzyme-solubilized collagen in a re-
action vessel maintained between pH 7 and 11 wherein
the fiberization and self-organization is likely to occur.
Examples of enzymes for solubilization include pepsin,
trypsin, chymotrypsin, papain, and pronase. Pepsin and
pronase are preferably used from the viewpoint of easy
handleability after the enzyme reaction.

3) A complex containing calcium salt and collagen

[0023] At least a part of the collagen in the complex
used in the present invention must be gelatinized. The
term "gelatin" refers to denatured collagen that is ob-
tained by cleavage of salt bonds or hydrogen bonds be-
tween peptide chains of collagen by treatment with boil-
ing water or the like, followed by irreversible denatura-
tion into a water-soluble protein. As mentioned above,
the term "collagen" used herein include this gelatinized
collagen. Gelatinization of collagen may be realized by
gelatinizing a part of the collagen constituting a complex
containing calcium salt and collagen. Alternatively, ge-
latinized collagen such as a commercialized solution of
gelatin powders may be externally added to the com-
plex.

[0024] In the production process according to the
present invention, a "complex containing calcium salt
and collagen" is employed as a starting material for the
reaction. Such a complex may be a commercialized one
or may be prepared in accordance with a known tech-
nique. A preferable example of the complex is a "com-
plex of hydroxyapatite and collagen." In particular, a
"self-organized complex containing hydroxyapatite and
collagen" is the most preferable. These complexes are
described in detail in 2.3 below.

1.2 Gelatinization of collagen

[0025] Examples of processes for gelatinizing a part
of the collagen constituting the complex containing cal-
cium salt and collagen include a method wherein a phys-
iological buffer such as PBS or Tris or water is added to
the complex, and the resultant is then heated, and a
method wherein a physiological buffer containing a mi-
nor amount of acid or water is added to the complex,
and the resultant is then heated.

[0026] The amount of a buffer or water to be added is
preferably about 24 to 60 ml per g of collagen (in the
case of a complex, 25% of which is constituted by col-
lagen, about 6 to 15 ml of buffer or water per g of the
complex). By changing the amount of the buffer added,
porosities, pore diameters, or strengths of the resulting
porous and composite materials can be adequately con-
trolled. When acid is added, a weak acid such as glu-
conic acid is preferably used together with a weak alkali
such as calcium carbonate in order to control the pH lev-
el. The amount of gluconic acid used is preferably about

collagen (in the case of a complex, constituted by collagen, about 200 mg of collagen per g of the complex). The acid is simultaneously added may be added in accordance with the amount of collagen to bring the pH level to around

is not added, the reaction temperature is between 40°C and 50°C, and particularly 45°C. The reaction time is preferably between 20 minutes. When acid is added, the reaction time is preferably between 35°C and 45°C, particularly around 40°C. The reaction time is preferably about 60 to 120 minutes. That the complex is gelatinized at relatively low temperature which is carried out with the addition

the reaction temperature or reaction time for gelatinization can be varied, and the mechanical strengths of the resulting composite materials can be adequately controlled. Specifically, if the reaction time is too short, gelatinization is incomplete. This results in small pore diameters and low mechanical strengths of the porous and composite materials. In contrast, if the reaction time is too long, excessive gelatinization occurs, resulting in a dense structure. This results in lowered strengths of the porous and composite materials. 1.3

ly, a complex containing collagen, which is gelatinized, is placed in an mold and allowed to freeze. The complex is placed in a mold, an additive is added to the complex in or-

der to introduce internal crosslinking between collagens constituting the complex. This is preferable since the resulting porous body has larger pore diameters and a larger number of open pores.

[0033] The term "internal crosslinking" used herein refers to crosslinking that is introduced between collagens that are present in the complex. Crosslinking occurs either between ungelatinized collagens, between gelatinized collagens, or between ungelatinized collagen and gelatinized collagen. Crosslinking may occur at any portions of collagens. Particularly preferable crosslinking occurs between a carboxyl group and a hydroxyl group, between a carboxyl group and a ϵ -amino group, or between ϵ -amino groups.

[0034] Any method, such as chemical crosslinking using a crosslinking agent or condensing agent or physical crosslinking using γ rays, ultraviolet rays, thermal dehydration, an electron beam, or the like, may be employed. Chemical crosslinking using a crosslinking agent is preferable.

[0035] Examples of crosslinking agents that can be used include: aldehyde crosslinking agents such as glutaraldehyde or formaldehyde; isocyanate crosslinking agents such as hexamethylene diisocyanate; carbodiimide crosslinking agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; polyepoxy crosslinking agents such as ethylene glycol diethyl ether; and transglutaminase, with glutaraldehyde being preferable. The amount of the crosslinking agent to be added is adequately determined in accordance with its type. In the case of glutaraldehyde, it is preferably 0.4 mg to 40 mg, and particularly preferably about 4 mg to 10 mg, per g of collagen before gelatinization (initial amount) (in the case of a complex, 25% of which is constituted by collagen, 0.1 mg to 10 mg, and particularly about 1 mg to 2.5 mg, per g of the complex).

3) Freezing

[0036] The cooled complex is allowed to freeze in accordance with a conventional technique. The freezing speed in a freezer is slowed in order to allow ice crystals to grow. These ice crystals become pores in the subsequent step of lyophilization. The pore sizes affect the strengths of the resulting porous and composite materials. Thus, the use of a refrigerator, the temperature of which can be subtly controlled, is more preferable from the viewpoint of the controllability of the freezing speed.

1.4 Lyophilization

[0037] The frozen complex is allowed to lyophilize in accordance with a conventional technique. During this step of lyophilization, numerous ice crystals, which were formed in the complex during the step of freezing, become pores. Thus, porous and composite materials having desirable porosities and pore diameters are obtained.

1.5 Surface crosslinking

[0038] The mechanical strengths of the resulting porous and composite materials can be enhanced by introducing surface crosslinking between collagens. The term "surface crosslinking" refers to crosslinking that is introduced between collagens that are present on the surface of the porous and composite materials.

[0039] Any method, such as chemical crosslinking using a crosslinking agent or condensing agent or physical crosslinking using γ rays, ultraviolet rays, thermal dehydration, an electron beam, or the like, may be employed for surface crosslinking. Examples of crosslinking agents include: aldehyde crosslinking agents such as glutaraldehyde or formaldehyde; isocyanate crosslinking agents such as hexamethylene diisocyanate; carbodiimide crosslinking agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; polyepoxy crosslinking agents such as ethylene glycol diethyl ether; and transglutaminase. The amount of the crosslinking agent to be used is adequately determined in accordance with its type. It is preferably at least 1 μ mol per g of the complex.

[0040] Crosslinking as mentioned above may occur at any portions of collagens. Particularly preferable crosslinking occurs between a carboxyl group and a hydroxyl group, between a carboxyl group and a ϵ -amino group, or between ϵ -amino groups. Crosslinking is preferably introduced in at least 1 %, and more preferably at least 5 %, of reactive functional groups. This is because sufficient mechanical strengths cannot be expected if crosslinking is insufficient. Attention should be given to the use of a crosslinking agent since the excessive use of a crosslinking agent disadvantageously embrittles a complex.

[0041] Among the aforementioned methods for crosslinking, chemical crosslinking using a crosslinking agent such as glutaraldehyde is particularly preferable from the viewpoints of controllability of the level of crosslinking and biocompatibility of the resulting complex. Chemical crosslinking can be performed by immersing the porous and composite materials obtained in the section above in a solution containing a crosslinking agent.

[0042] A method for crosslinking using glutaraldehyde is hereafter described as a preferable embodiment of the present invention. At the outset, the porous and composite materials obtained in the section above are immersed in a solution containing 0.001% to 1%, and preferably about 0.005% to 0.1%, glutaraldehyde (degassing and aspiration). Use of 50% to 100% ethanol as a solvent is preferable since crosslinking density is enhanced. Conditions for immersion are adequately determined in accordance with the concentration of glutaraldehyde. Preferably, it is generally slowly carried out over the course of a period of approximately 60 minutes to 5 hours at room temperature or lower temperature in order to allow the crosslinking to homogeneously occur.

After the crosslinking, the porous and composite materials are washed with pure water to remove excess glutaraldehyde. Further, remaining glutaraldehyde may be preferably neutralized with the use of a glycine solution or the like. The porous and composite materials may be further lyophilized after crosslinking.

[0043] Thus, the porous and composite materials can be provided with desirable mechanical strengths by introducing surface crosslinking therein. The degradation rate in an organism can be controlled based on the level of crosslinking to be introduced.

1.6 Others

[0044] In addition, adequate modifications or additional steps can be added to the aforementioned steps within the scope of the present invention. For example, addition of polysaccharides such as hyaluronic acid prior to the crosslinking can increase the points of crosslinking and enhance the mechanical strengths. Alternatively, polysaccharides such as hyaluronic acid can be added instead of gelatinized collagen, crosslinking can be introduced thereto, and the resultant can be allowed to gelatinize and freeze. Thus, a porous body having large pore diameters and high porosities can be obtained.

[0045] A wide variety of treatments can be applied in order to enhance the strengths of the porous and composite materials after surface crosslinking. For example, the surfaces of the porous and composite materials may be coated with hydroxyapatite and collagen by immersing them in a slurry of a complex of hydroxyapatite and collagen. The surfaces of the porous and composite materials may be coated with hydroxyapatite by immersing them in a slurry of hydroxyapatite. Alternatively, hydroxyapatite may be precipitated not only on the surfaces but also on the insides of the porous and composite materials by alternate immersion in calcium and phosphorus.

2. The porous and composite materials of the present invention

2.1 Properties of the porous and composite materials (porosities; pore diameters, and mechanical strengths)

[0046] The porous and composite materials obtained by the method according to the present invention have high porosities, large pore diameters, and adequate mechanical strengths. The porosities of the porous and composite materials obtained under preferable conditions are 80% to 95%, and the pore diameters thereof are about 50 μ m to 2,000 μ m. However, the porosities or pore diameters of the porous and composite materials can be adequately changed by altering the amount of a buffer, the reaction temperature, and the reaction time in the step of gelatinization, the freezing speed in the step of freezing, or other factors. Accordingly, porous and composite materials having desirable porosities and pore diameters can be obtained by changing the aforementioned conditions. Further, conditions for

crosslinking can be adequately de-
 rous and composite materials hav-
 ts.

Properties of porous and composite 5

ies and the pore diameters of the
 e materials of the present invention
 d evaluated by, for example, image
 micrographs. Mechanical strengths
 ed on, for example, the three-point
 the Young's modulus. determined
 natively, the strength can be eval-
 uational analysis of operability when
 ch as during transplantation tests.

porous and composite materials
 ate and collagen

e example of the porous and com-
 present invention is a microporous
 rial containing hydroxyapatite and
 c-axis of hydroxyapatite is oriented
 ber.

site material is produced using a
 ex containing hydroxyapatite and
 g material. The term "self-organ-
 to the "formation of a specific or-
 ne aggregation of homologous or
 molecules, fine particles, or the like
 "ing interaction" (Seikagaku Jiten
 misty), Tokyo Kagaku Dozin Co.,
 invention, this term particularly re-
 hydroxyapatite having an apatite
 entation that is specific to biologi-
 gen fibers. That is, a microporous
 c-axis of hydroxyapatite is orient-
 ers is formed. Self-organized com-
 droxyapatite and collagen can be
 ce with, for example, the method
 chi, S. et al., J. Biomater., 22 (13)
 Itoh et al., J. Biomed Mater Res.

porous structure in which the c-axis
 oriented along collagen fibers is par-
 the production step according
 on. Accordingly, the resulting po-
 materials have microporous struc-
 of biological bones.

and composite materials of the
 contain other components within
 ent invention. Examples of such
 organic salts such as St, Mg, and
 ces such as citric acid and phos-

pholipids, Bone Morphogenetic Proteins, and agents
 such as an anti-cancer agent.

3. A method for utilizing porous and composite materials

3.1 Implants

[0052] The porous and composite materials obtained
 by the method of the present invention have high poros-
 ities, and each component thereof is biodegradable. Ac-
 cordingly, they are easily penetrated by cells and they
 are excellent in bone conductivity because they contain
 hydroxyapatite and collagen. Further, adequate me-
 chanical strengths and retentivity in organisms (ade-
 quate rate of biodegradation) can be controlled by
 crosslinking. Accordingly, the porous and composite
 materials of the present invention are suitable for im-
 plants that are used in the field of orthopedics such as
 bone fillers or implants that are used in the field of den-
 tistry.

[0053] The configurations and forms of the aforemen-
 tioned implants are not particularly limited. Implants can
 take any desired configurations and forms in accord-
 ance with their applications. For example, they can be
 blocks, pastes, films, particles, or sponges.

[0054] The porous and composite materials of the
 present invention have low compressive strengths. Up-
 on moisture absorption, they become elastic as sponges,
 they are less likely to collapse even with the appli-
 cation of forces strong enough to deform (crush) them,
 they reabsorb moisture, and they rapidly regain their
 original forms. This feature results in an excellent oper-
 ability at the time of surgical operations. When the po-
 rous and composite materials of the present invention
 are used as implants by making use of the aforemen-
 tioned feature, they may be once immersed in an ade-
 quate liquid such as physiological saline before use.

3.2 Drug carrier for sustained-release (cell carrier, DNA
 carrier)

[0055] The porous and composite materials of the
 present invention have large surface areas of hydroxya-
 patite or the like that are capable of adsorbing a protein,
 DNA, or the like and the densities thereof are not too
 high. Thus, penetration and release of an agent or the
 like can easily occur. Accordingly, the porous and com-
 posite materials of the present invention can be used as
 a drug carrier for sustained-release. Drug carriers for
 sustained-release using the porous and composite ma-
 terials of the present invention have high drug adsorp-
 tivity, and their forms are well preserved. The aforemen-
 tioned drug carriers for sustained-release have higher
 drug-adsorptivity and penetrability by cells compared
 with a conventional block of hydroxyapatite and colla-
 gen. The drug carriers for sustained-release using the
 porous and composite materials of the present invention
 can prevent carcinoma recurrence and induce the gen-

4. Examination of conditions for freezing

[0078] The HAp/Col porous body was prepared in the
 same manner as in Example 1 except that the HAp/Col
 complex was allowed to freeze in a freezer at -80°C
 without being placed in a case in the step of freezing.
 The resultant was compared with the HAp/Col porous
 body obtained in Example 1 (Fig. 6).

[0079] As a result, the porous body prepared by freez-
 ing at -80°C had very small pore diameters compared
 with that prepared by freezing at -20°C.

[Example 3] Experimentation for transplanting the HAp/
 Col porous body to a rat thighbone

1. Preparation of implants for transplantation

[0080] In accordance with the method of Kikuchi et al.,
 500 mg of HAp/Col complex powders (HAp:Col = 3:1)
 were prepared. PBS (4 ml), 300 mg of gluconic acid,
 and 83 mg of calcium carbonate were added thereto,
 and the mixture was incubated at 40°C for 90 minutes
 for gelatinization. The gelatinized complex was allowed
 to freeze in a freezer at -20°C, and further lyophilized to
 obtain the HAp/Col porous body. The resulting porous
 body was immersed in a solution of 0.01 % glutaralde-
 hyde for 2 hours to introduce crosslinking between col-
 lagens on the surface of the complex (surface crosslink-
 ing).

[0081] Porous bodies were prepared under the above
 conditions. One type thereof was provided with internal
 crosslinking in the same manner as in Example 1, and
 another type was provided with surface crosslinking in-
 troduced with 0.5 % glutaraldehyde (GA).

[0082] The prepared porous bodies were cut into 2 x
 2 x 3 mm portions to prepare implants to be transplanted
 into rat thighbones.

2. Transplantation into rat thighbones

[0083] A hole (diameter: approximately 3 mm) was
 provided in the distal part of a Wistar rat thighbone, and
 the prepared implants were transplanted therein. The
 implants were taken out 1, 2, 3, 4, and 6 weeks after the
 transplantation and stained with hematoxylin and eosin
 for observation (Figs. 7 to 12).

3. Results

(1) HAp/Col porous body surface-crosslinked with 0.01
 % GA

[0084] This porous body had lower porosity and a
 smaller number of continuous pores compared with the
 porous body of (2), which was subjected to internal
 crosslinking. Thus, penetrability by cells was less suffi-
 cient. With the elapse of time, however, implants were
 absorbed, and good bone formation was observed.

[0085] One week after the transplantation, good cell
 penetration in the vicinity of formation of neonatal bones
 was observed in a part thereof (Figs. 7B, 7C). In other
 parts, there were pores which cells did not penetrate
 (Fig. 7A). Cell penetration was enhanced 2 weeks after
 the transplantation, and formation of neonatal bones
 that were in contact with the implants was observed.
 Three weeks after the transplantation, cell penetration
 and formation of neonatal bones were further enhanced
 in parenchyma (Figs. 8A, 8C), and implants were signif-
 icantly decreased because of absorption as neonatal
 bones were formed (Fig. 8B).

(2) HAp/Col porous body surface-crosslinked with 0.01
 % GA and internally crosslinked

[0086] The porous body to which internal crosslinking
 had been applied had somewhat larger pore diameters
 and the penetration by cells was better compared with
 the porous body of (1). While good cell penetration and
 formation of neonatal bones around the implants were
 observed 2 weeks after the transplantation (Fig. 9A), ab-
 sorption of implants by osteoclasts was observed (Fig.
 9B). Four weeks after the transplantation, absorption of
 implants and neonatal bones was observed (Fig. 10A to
 10C). It was observed that absorption of implants and
 neonatal bones was further enhanced 6 weeks after the
 transplantation (Fig. 11A to 11C).

(3) HAp/Col porous body surface-crosslinked with
 0.05% GA

[0087] This porous body had frequent crosslinking.
 Thus, absorption of implants was poor, and implants still
 remained even 6 weeks after the transplantation (Fig.
 12D).

[0088] All publications, patents, and patent applica-
 tions cited herein are incorporated herein by reference
 in their entirety.

Industrial Applicability

[0089] The present invention enables the production
 of biodegradable porous and composite materials which
 have large pore diameters, high porosities, and me-
 chanical strengths that are suitable for alternative bone
 implants. The porous and composite materials pro-
 duced by the present invention are very useful as im-
 plants, various carriers such as drug carriers for sus-
 tained-release, and supports for cell culture.

Claims

1. A process for producing porous and composite ma-
 terials comprising steps of:

freezing a complex containing at least one cal-

lected from calcium carbonate, calcium phosphate, and hydroxyapatite and collagen, at least a part of which is gelatinized; and lyophilizing the resultant.

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producing porous and composite materials by introducing surface crosslinking between collagens to the porous and composite materials obtained by the process according to claim 1.

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According to claim 2, wherein the step of surface crosslinking is carried out by introducing surface crosslinking agents to the porous and composite materials in a solution containing a crosslinking agent.

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According to any one of claims 1 to 3, the step of adding a crosslinking agent to the porous and composite materials is to introduce internal crosslinking between collagens.

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According to any one of claims 1 to 4, the calcium salt is hydroxyapatite.

producing porous and composite materials by the following steps of:

25

extracting at least a part of the collagen from a complex containing hydroxyapatite and collagen;

30

introducing internal crosslinking between the collagen with the addition of a crosslinking agent to form a complex;

producing porous and composite materials by introducing surface crosslinking between the complex and then lyophilizing the resultant.

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According to any one of claims 2 to 6, the step of surface crosslinking between the porous and composite materials is carried out by immersing the porous and composite materials in a solution containing a crosslinking agent.

40

According to any one of claims 2 to 6, the step of surface crosslinking between the porous and composite materials is carried out by introducing glutaraldehyde as a crosslinking agent.

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According to any one of claims 1 to 7, the porous and composite materials have porosities of 80% or higher.

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The porous and composite materials having porosities of 80% or higher which are produced by the method according to any one of claims 1 to 8.

The porous and composite materials according to any one of claims 1 to 8.

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Organisation für geistiges Eigentum
Internationales Büro



D2

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Postfachstrasse 27, 64407 Fränkisch-Crumbach
KE, Brigitte [DE/DE]; Rosserstrasse 33,
Frankfurt (DE). SCHAFFNER, Patricia [DE/DE];
Postfach 15, 64347 Griesheim (DE).

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

STRUCTURED COMPOSITES AS A MATRIX (SCAFFOLD) FOR THE TISSUE ENGINEERING OF BONES

STRUKTURIERTE KOMPOSITE ALS MATRIX (SCAFFOLD) FÜR DAS TISSUE ENGINEERING VON

The invention relates to a structured composite as a carrier for the tissue engineering and implant material of bones, consisting of porous calcium phosphate granulates. The granulated material has a grain size of between 0.5 and 10 mm micropores, and the macropores have an average diameter of between 50 and 500 µm and are bound by a binding agent.

Erfindung: Gegenstand der Erfindung ist ein strukturiertes Komposit als Träger für das Tissue Engineering und Implantatmaterial von Knochen bestehend aus einer Schüttung von porösen Calciumphosphat-Granulaten, wobei das Granulat eine Korngröße von 0,5 bis 10 mm mit Makro- und Mikroporen besitzt, die Makroporen einen mittleren Durchmesser von 50 bis 500 µm haben und durch ein bioverträgliches Bindemittel gebunden sind.

Strukturierte Komposite als Matrix (Scaffold) für das Tissue Engineering von Knochen

Die Erfindung beschreibt ein strukturiertes Komposit als Träger für das Tissue Engineering und Implantatmaterial von Knochen (in situ und in vitro) bestehend aus einer Schüttung von porösen Calciumphosphat-Granulaten mit einer Korngröße von 0.5 bis 10 mm mit Makro- und Mikroporen, wobei die Makroporen einen mittleren Durchmesser von 50 bis 500 µm haben und durch ein bioverträgliches Bindemittel gebunden sind.

Synthetischer Ersatz für Eigenknochen oder Spenderknochen gewinnt zunehmend an Bedeutung, weil einerseits die Qualität der entwickelten Materialien zunehmend die Anforderungen der Kunden erfüllt und andererseits die mit den biologischen Materialien verbundenen Nachteile vermieden werden, wie insbesondere eingeschränkte Verfügbarkeit, Entnahmeprobleme beim Eigenknochen, Risiko der Krankheitsübertragung beim Spenderknochen.

Um die biologische Wertigkeit der synthetischen Materialien zu erhöhen, gibt es die Ansätze, den Knochenersatz mit rekombinanten Wachstumsfaktoren, autologen Wachstumsfaktoren (Thrombozyten-Präparationen), Adhäsionsfaktoren (RGD-Peptide) und/oder autologen Zellpräparationen (Knochenmarkaspirat) aufzuwerten.

Um die gewünschten Eigenschaften von optimierten Knochenersatzmaterialien zu erhalten, gibt es vor allem zwei Entwicklungsschwerpunkte, einmal die mechanische Optimierung für lasttragende Anwendungen bzw. strukturelle Implantate und zum anderen die biologische Optimierung mit dem Ziel einer möglichst schnellen und vollständigen Regeneration von vollwertigen Knochengewebe (während dabei die mechanische Funktion von Metallimplantaten übernommen wird).

Herstellungsbeispiel:**Herstellung**

Das Komposit kann in unterschiedlichen Größen hergestellt werden. Aus einem Gemisch von 30 g Collagen-Suspension mit 7g Calciumphosphat wird ein Komposit mit der Größe 8 x 2 cm mit 0.7 cm Dicke hergestellt. Das Gemisch wird über Nacht tiefgefroren, dann kurzzeitig aufgetaut. Im Anschluss findet die Gefriertrocknung des Gemisches unter Standardbedingungen statt. Standardbedingungen bedeutet: Einfrierphase (-20°C, 1-3 Std.), Erwärmungsphase (0°C, 1-3 Std.), Trocknungsphase (0-10°C, 12-18 Std.)
Das hergestellte Produkt enthält 98 bis 99 Gew. % Calciumphosphat und 1 bis 2 Gew. % Collagen.

Verwendung mit Blut:

Das hergestellte Komposit zeigt hervorragende Eigenschaften bei der Verwendung mit Blut. Es saugt das Blut vollständig auf. Es zeigt sich keine Quellung oder Verformung des Komposits.

Stabilität

Die Stabilität in Puffer kann über zwölf Tage nachgewiesen werden. Nach Ablauf dieser zwölf Tage bleibt das Komposit kompakt und die Granula sind zusammengehalten.

Patentansprüche

1. Strukturiertes Komposit als Träger für das Tissue Engineering und Implantatmaterial von Knochen bestehend aus einer Schüttung von porösen Calciumphosphat-Granulaten, dadurch gekennzeichnet, dass das Granulat eine Korngröße von 0.5 bis 10 mm mit Makro- und Mikroporen besitzt, wobei die Makroporen einen mittleren Durchmesser von 50 bis 500 µm haben, gebunden durch ein bioverträgliches Bindemittel.
2. Komposit nach Anspruch 1, dadurch gekennzeichnet, dass die Mikroporen einen mittleren Durchmesser von 0.5 bis 5 µm besitzen.
3. Komposit nach den Ansprüchen 1 und/oder 2, dadurch gekennzeichnet, dass die Größe der Granulatkörner 1 bis 4 mm beträgt.
4. Komposit nach einem der Ansprüche 1 bis 3, dadurch gekennzeichnet, dass das Bindemittel Kollagen, vernetzte Gelatine oder Hyaluronsäure ist.
5. Komposit nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass es aus 0.2 bis 10 Gew. % Bindemittel, bezogen auf das Komposit, besteht.
6. Komposit nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass es aus 0.2 bis 2 Gew. % Bindemittel, bezogen auf das Komposit, besteht.
7. Komposit nach einem der Ansprüche 1 bis 6, dadurch gekennzeichnet, dass das Bindemittel Kollagen aus Typ I -Kollagen oder Mischungen aus Typ I und III besteht.

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posit nach einen der Ansprüche 1 bis 7, dadurch gekennzeichnet,
s das Bindemittel vernetzt wird.

posit nach einen der Ansprüche 1 bis 8, dadurch gekennzeichnet,
s es mit autologen Faktoren wie Wachstumsfaktoren,
ombozytenpräparationen und/oder autologe Zellpräparationen
/oder Zellextrakte/Proteinpräparationen beladen ist.

posit nach einen der Ansprüche 1 bis 8, dadurch gekennzeichnet,
s es mit rekombinanten Faktoren oder deren Teilstücke wie
chstumsfaktoren, Adhäsionsproteine und/oder Adhäsionspeptiden
aden ist.

posit nach einen der Ansprüche 1 bis 8, dadurch gekennzeichnet,
s es mit Antibiotika beladen ist.

fahren zur Herstellung eines strukturierten Komposits als Träger für
Tissue Engineering und Implantatmaterial von Knochen,
ennzeichnet durch folgende Merkmale:

chen einer Lösung oder Suspension von Bindemittel mit
ciumphosphat-Granulat mit einer Korngröße von 0.5 bis 10 mm mit
ro- und Mikroporen, wobei die Makroporen einen mittleren
chmesser von 50 bis 500 µm haben;
chließend tiefgefrieren und auftauen sowie Gefriertrocknung des
nisches unter Standardbedingungen.

fahren nach Anspruch 12, dadurch gekennzeichnet, dass das
demittel nachfolgend chemisch vernetzt wird.

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料研究機構内 Ibaraki (JP). 庄司 大助 (SHOJI, Daisuke) [JP/JP]; 〒174-8639 東京都板橋区前野町 2 丁目 36 番 9 号 ペンタックス株式会社内 Tokyo (JP).

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(74) 代理人: 高石 橋馬 (TAKAISHI, Kitsuma); 〒162-0825 東京都新宿区神楽坂 6 丁目 67 神楽坂 FN ビル 5 階 Tokyo (JP).

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(71) 出願人 (米国を除く全ての指定国について): 独立行政法人物質・材料研究機構 (NATIONAL INSTITUTE FOR MATERIALS SCIENCE) [JP/JP]; 〒305-0047 茨城県つくば市千現 1 丁目 2-1 Ibaraki (JP). ペンタックス株式会社 (PENTAX CORPORATION) [JP/JP]; 〒174-8639 東京都板橋区前野町 2 丁目 36 番 9 号 Tokyo (JP).

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(72) 発明者; および

(75) 発明者/出願人 (米国についてのみ): 菊池 正紀 (KIKUCHI, Masanori) [JP/JP]; 〒305-0047 茨城県つくば市千現 1 丁目 2-1 独立行政法人物質・材料研究機構内 Ibaraki (JP). 田中 順三 (TANAKA, Junzo) [JP/JP]; 〒305-0047 茨城県つくば市千現 1 丁目 2-1 独立行政法人物質・材料研究機構内 Ibaraki (JP). 小林 尚俊 (KOBAYASHI, Hisatoshi) [JP/JP]; 〒305-0047 茨城県つくば市千現 1 丁目 2-1 独立行政法人物質・材

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(54) Title: APATITE/COLLAGEN CROSSLINKED POROUS MATERIAL CONTAINING SELF-ORGANIZED APATITE/COLLAGEN COMPOSITE AND PROCESS FOR PRODUCING THE SAME

(54) 発明の名称: 自己組織化したアパタイト/コラーゲン複合体を含むアパタイト/コラーゲン架橋多孔体及びその製造方法

(57) Abstract: A process for producing an apatite/collagen crosslinked porous material, comprising gelling a dispersion containing an apatite/collagen composite and collagen, lyophilizing the gel to thereby obtain a porous body and crosslinking the collagen of the porous body; and an apatite/collagen crosslinked porous material produced by this process.

(57) 要約: アパタイト/コラーゲン複合体とコラーゲンとを含む分散物をゲル化した後で凍結乾燥することにより多孔質体とし、次いで多孔質体中のコラーゲンを架橋することによりアパタイト/コラーゲン架橋多孔体を製造する方法、及びこの方法により得られたアパタイト/コラーゲン架橋多孔体。

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Extension States:

- TANAKA, J., National Inst. for Materials Science
Tsukuba-shi, Ibaraki 305-0047 (JP)
- KOBAYASHI, H,
National Inst. for Materials Science
Tsukuba-shi, Ibaraki 305-0047 (JP)
- SHOJI, Daisuke, c/o Pentax Corporation
Itabashi-ku, Tokyo 174-8639 (JP)

2002 JP 2002322507

ute for Materials Science
47 (JP)
ation
9 (JP)

- (74) Representative:
Schaumburg, Thoenes, Thurn, Landskron
Patentanwälte
Postfach 86 07 48
81634 München (DE)

ational Inst. for Materials Science
baraki 305-0047 (JP)

COLLAGEN CROSSLINKED POROUS MATERIAL CONTAINING SELF-ORGANIZED
COLLAGEN COMPOSITE AND PROCESS FOR PRODUCING THE SAME

producing a cross-linked apatite/
y comprising the steps of gelling
g a dispersion containing an apa-
te and collagen to form a porous

body, and further cross-linking collagen in the porous
body, and a cross-linked apatite/collagen porous body
produced by this method.

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a cross-linked
apatite/collagen porous body containing an apatite/colla-
gen composite for use in artificial bone, cell scaffold,
etc., and its production method.

BACKGROUND OF THE INVENTION

[0002] Because of excellent compatibility with human
bone, artificial bone made of apatite can be bonded to
the human bone directly. Accordingly, the artificial bone
made of apatite has recently been appreciated for effec-
tiveness, finding clinical applications in cosmetic sur-
gery, neurosurgery, plastic surgery, oral surgery, etc.
However, artificial ceramic bone such as apatite is not
necessarily completely identical with human bone in
terms of mechanical properties and physiological prop-
erties. For instance, a so-called artificial ceramic bone
made only of apatite is harder and more brittle than the
human bone. While the human bone is repeatedly sub-
jected to metabolism of absorption and regeneration,
the artificial bone made of apatite is not substantially dis-
solved but semi-permanently remains in human body.
The remaining artificial bone breaks human bone at an
interface with the human bone, making it likely to cause
bone fracture.

[0003] Research has recently become active on arti-
ficial bone decomposable in the human body, which is
closer in composition to human bone than the artificial
apatite bone, and various proposals have been made.
For instance, JP 11-513590 A discloses a cross-linked
apatite/collagen porous body having a network struc-
ture, in which collagen and, if necessary, other binders
are bonded to hydroxyapatite. Because this cross-
linked apatite/collagen porous body is decomposable in
human body, human bone is formed in the cross-linked
apatite/collagen porous body, and the cross-linked apa-
tite/collagen porous body per se is absorbed in a hu-
man body. Accordingly, this cross-linked apatite/colla-
gen porous body can be used for the fixation of vertebra,
the filling of bone defects, the repair of fractured bone
and, the grafting of periodontal defects, etc. However,
this cross-linked apatite/collagen porous body is a sim-
ple mixture of collagen and apatite, which does not have
a living human bone structure, in which the C-axis of the
apatite is oriented along collagen fibers. Further, this
cross-linked apatite/collagen porous body has insuffi-
cient mechanical strength and poor bone neoplasia.

OBJECT OF THE INVENTION

[0004] Accordingly, an object of the present invention
is to provide a cross-linked apatite/collagen porous body
absorbed in a human body according to the same mech-
anism as in living human bone, and having high bone

neoplasia, which can be used for artificial bone, etc., and
its production method.

DISCLOSURE OF THE INVENTION

[0005] As a result of intense research in view of the
above object, the inventors have found that when a dis-
persion containing an apatite/collagen composite and
collagen is gelled before its freeze-drying, the resultant
cross-linked apatite/collagen porous body has a uniform
structure with high strength. The present invention has
been completed based on this finding.

[0006] Thus, the method for producing a cross-linked
apatite/collagen porous body according to the present
invention comprises the steps of freeze-drying a disper-
sion containing an apatite/collagen composite and colla-
gen to form a porous body, and then cross-linking colla-
gen in the porous body, the dispersion being freeze-
dried after gelation.

[0007] A ratio of the apatite/collagen composite to the
collagen is preferably 97/3 to 93/7 by mass in the dis-
persion. An apatite/collagen ratio is preferably 9/1 to 6/4
by mass in the apatite/collagen composite.

[0008] In the gelation of the dispersion, the dispersion
temperature is preferably kept at 35 to 43°C, and the
dispersion preferably has pH of 6.8 to 7.6 and ionic
strength of 0.2 to 0.8 before the gelation.

[0009] The apatite/collagen composite is preferably in
the form of fibers as long as 0.01 to 1 mm. Apatite in the
apatite/collagen composite preferably has a C-axis ori-
ented along the collagen fibers. Apatite in the cross-
linked apatite/collagen porous body also preferably has
a C-axis oriented along the collagen fibers.

[0010] The cross-linked apatite/collagen porous body
of the present invention is produced by any of the above
methods.

BEST MODE FOR CARRYING OUT THE INVENTION

[1] Production of apatite/collagen composite

[0011] In the production of the cross-linked apatite/
collagen porous body of the present invention, an apa-
tite/collagen composite and collagen as a binder are
used as starting materials. The apatite/collagen com-
posite preferably has a structure similar to that of a living
human bone, in which hydroxyapatite and collagen are
oriented in a self-organized manner. The term "self-or-
ganization" means that calcium hydroxyphosphate (hy-
droxyapatite) having an apatite structure has orientation
peculiar to living human bone along collagen fibers,
namely that the C-axis of the hydroxyapatite is in align-
ment with the collagen fibers.

(1) Starting materials

[0012] The apatite/collagen composite is produced
from collagen, phosphates and calcium salts as starting



ates Patent [19]

[11] Patent Number: 5,320,844

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E MATERIALS FOR HARD
PLACEMENT

ung-Tsuen Liu, 29 Landing, Laguna
iguell, Calif. 92677

49,880

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Primary Examiner—Thurman K. Page
Assistant Examiner—Peter F. Kullosky
Attorney, Agent, or Firm—Frank J. Uxa

[57] ABSTRACT

Hard tissue replacement materials and methods for making same are disclosed. In one embodiment, the present composition comprises a synthetically derived, substantially homogenous composite containing collagen component and calcium phosphate-containing component precipitated from a liquid medium in which the collagen component is contained.

17 Claims, No Drawings

COMPOSITE MATERIALS FOR HARD TISSUE
REPLACEMENT

BACKGROUND OF THE INVENTION

This invention relates to composite materials for hard tissue replacement, and to methods for producing such materials. More particularly, the invention relates to collagen-containing composites, substrates coated with calcium, phosphate-containing components and methods for producing same. The collagen-containing composite materials can be prepared by precipitation of calcium, phosphate-containing materials, such as calcium apatite materials, in a collagen slurry. The coated calcium, phosphate-containing substrates can be prepared by contacting an uncoated substrate in a supersaturated or oversaturated solution of coating materials, such as calcium apatites, to coat the surface of the substrate.

The composition of hard tissue, such as natural bone, comprises collagen and inorganic calcium phosphate, particularly biological apatite. Bone contains about 60% to 75% by weight of biological apatite and tooth has more than 98% by weight of biological apatite. Biological apatite is a naturally occurring calcium apatite-type material which is formed in the body by precipitation from body fluids at body conditions. This biological apatite has a structure which is similar to pure hydroxyapatite, but contains some substitute ions for the calcium, phosphate and hydroxyl ions. Strictly speaking, synthetically produced precipitated hydroxyapatite is more similar to biological apatite than are the hydroxyapatite ceramics.

Synthetically produced precipitated hydroxyapatite is a very fine powder. It has limited application as a hard tissue implant material because it is somewhat difficult to manipulate.

Recently, premolded collagen incorporated with a granule form of hydroxyapatite ceramic (HA) have been commercialized. In such collagen-HA composites, the collagen and hydroxyapatite ceramics form a completely heterogeneous mixture. In effect, the collagen serves as the binder system for the granules of the hydroxyapatite ceramics. Such heterogeneous composite materials are completely different from the natural bone. It would be advantageous to provide a hard tissue replacement material which has an increased similarity to natural bone.

In the last decade or so, many calcium phosphate-containing ceramics and glasses have been prepared in granule form or block form for hard tissue substitute materials. Among these are hydroxyapatite ceramics, tricalcium phosphate ceramics and calcium, phosphate-containing bioglasses. Clinical studies have confirmed that hydroxyapatite ceramics and such bioglasses are very good biocompatible materials for use as artificial hard tissue implant materials. After implantation, hydroxyapatite ceramics and calcium, phosphate-containing bioglasses bond very strongly with bone.

The hydroxyapatite ceramics, as well as the bioglasses, are somewhat different from biological apatite. In general, hydroxyapatite ceramics and bioglasses after implantation take a certain amount of time to adjust to the body's environment. Biological apatite from new bone starts to grow and bind to the surface of these implant materials. For example, calcium, phosphate-containing bioglass, after soaking in body fluid, takes several weeks to form a precipitated hydroxyapatite

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surface layer. The time for the formation of a hydroxyapatite layer depends strongly on the composition of the bioglass. Clinical studies have showed that the bonding strength between the bioglass and the bone during the initial healing stage also depends on the rate of formation of this hydroxyapatite surface layer. In general, those bioglasses with a fast rate of formation of precipitated surface hydroxyapatite also show a strong bonding with bone. Even though untreated hydroxyapatite ceramics and bioglasses have excellent biocompatibility, it would be advantageous to provide additional biocompatibility and to provide hard tissue replacement materials which shorten the healing time.

SUMMARY OF INVENTION

New hard tissue implant materials and methods for producing same have been discovered. The present invention provides collagen-containing composite materials which have properties generally similar to natural bone. Moreover, these synthetic composite materials can be produced so as to substantially eliminate the risk of immune system reactions, which often cause implant failure if the implant is made of natural bone matter. Further, the present materials which comprise coated substrates facilitate healing, for example, reduce the healing time required relative to the use of uncoated implant materials or substrates, from implantation surgery.

In one broad aspect, the present compositions, which are useful as hard tissue replacement or implant materials, comprise synthetically derived, substantially homogenous composites containing collagen component and calcium, phosphate-containing component precipitated from a liquid medium in which the collagen component is contained. Methods for producing such implant materials are also disclosed and are within the scope of the present invention.

In another broad aspect of the invention, compositions, which are useful as hard tissue replacement or implant materials, comprise a substrate, preferably selected from calcium apatite ceramics, calcium, phosphate-containing bioglasses and mixtures thereof, and a precipitated coating material selected from calcium apatites (meaning to include therein, for example, calcium hydroxyapatite, calcium fluorapatite, calcium carbonateapatite and the like) and mixtures thereof deposited on at least a portion of the external surfaces of the substrate from a supersaturated or oversaturated solution of the coating material prior to the composition being implanted in the body of an animal. Methods for producing such implant materials are also disclosed and are within the scope of the present invention.

DETAILED DESCRIPTION OF THE
INVENTION

For the preparation of the present collagen component, calcium, phosphate-containing component composites, any suitable collagen component may be employed. The preferred collagen component used in the present invention is type I collagen. In one embodiment, the solid collagen component is dispersed in an acidic liquid, preferably aqueous, medium to form a collagen component-containing slurry, for example, in the form of a gel. The concentration of collagen component in this slurry is preferably in the range of about 0.5% to about 3% by weight. Either a calcium-containing component, e.g., salt, or a phosphate-containing

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it, may be combined in, preferably collagen slurry. If a calcium-containing component is combined into the collagen slurry, the calcium component is separately combined, dissolved in, a liquid medium, preferably to form a solution. Similarly, if a phosphate-containing component is combined into the calcium-containing component, preferably dissolved in, a liquid medium, preferably to form a solution. Alternatively, two separate liquid media, preferably with the calcium-containing component and the phosphate-containing component, may be combined.

The slurry and the liquid medium are combined to a level of about 7 or higher, preferably. If the slurry and two liquid media containing the calcium-containing component and the phosphate-containing component, the two liquid media (and the liquid media) are preferably adjusted to a higher, more preferably to a pH of 7 or higher. In this instance, the slurry itself is made acidic to better disperse the collagen. (or media) are then combined. Before and during this mixing, the combination or mixture are preferred over strong stirring, for example, or other conventional mixing medium is (or liquid media are) preferred to the stirred slurry. This mixing is most effective to cause the formation of a calcium, phosphate-containing complex, hydroxyapatite.

To limit the invention to any particular, it is believed that the precipitated calcium-containing component is an integral component to form a substantial material. After the mixing is complete, the collagen-containing material is dried, for example, by being filtered and/or washed several times with water; of other components, such as insoluble impurities. The wet solid, when shaped and dried, for example, air drying, oven drying and the like composite material or a porous enhance the mechanical strength of collagen cross-linking reagent can vary before separation and purification. A cross-linking agent is employed, for a sufficient period of time at to obtain the desired cross-linking agent.

The precipitation of a calcium apatite from the collagen slurry, the preferred to phosphate is about 1 to about 2, at 1.67. However, other mole ratios of such other mole ratios of Ca to P, when the pH of the combined media is about 10 or higher.

In component, calcium, phosphate containing composites may have widely varied provided that such composites are used application. Preferably, such composites about 25% to about 95% by weight component and about 5% to about 25% calcium, phosphate-containing com-

ponent. The calcium, phosphate-containing component included in the present composites is preferably selected from calcium phosphates, calcium apatites (meaning to include therein calcium hydroxyapatite, calcium fluorapatite, calcium carbonateapatite and the like apatite-type materials) and mixtures thereof. Composites including calcium fluorapatite can be produced by including fluoride (fluoride ion) in one or more of the materials used to produce the present composites. The present composites may include one or more additional components useful to provide one or more desired properties to the composites. Metals, such as alkali metals, other alkaline earth metals and the like, may replace at least a portion, for example, a minor portion, of the calcium. At least a portion, for example, a minor portion, of the phosphate and/or hydroxyl contents of the calcium, phosphate-containing material may be replaced by halogens, such as chloride, fluoride and the like, carbonate and the like. The precipitated calcium, phosphate-containing material can have a chemical composition which resembles or even simulates that of biological apatite. Different components may be added to one or more of the materials (e.g., slurry and liquid medium or media) used to make the present composites to adjust the chemical composition of the product composite, as desired.

Any suitable, preferably soluble and more preferably water soluble, calcium-containing component may be employed in the present methods for making collagen-containing composites, provided that such calcium-containing component functions as described herein and results in a useful collagen-containing composite in accordance with the present invention. Such calcium-containing component is preferably substantially free of phosphorus. Calcium-containing salts and mixtures thereof are particularly useful. Examples of calcium-containing salts include calcium acetate, other soluble calcium carboxylates, calcium chloride, calcium nitrate, other soluble calcium organic salts and mixtures thereof.

Any suitable, preferably soluble and more preferably water soluble, phosphate-containing component may be employed in the present methods for making collagen-containing composites, provided that such phosphate-containing component functions as described herein and results in a useful collagen-containing composite in accordance with the present invention. Such phosphate-containing component is preferably substantially free of calcium. Phosphate-containing salts and mixtures thereof are particularly useful. Examples of phosphate-containing salts include alkali metal phosphate salts, ammonium phosphate salt and mixtures thereof.

While any suitable liquid medium may be employed (for example, for the slurry and/or the solution or solutions), the use of aqueous liquid media is much preferred. Various different materials may be employed to maintain or adjust the pH of the slurry and/or liquid medium or media, as desired. Examples of useful pH adjusting agents include alkali metal hydroxides, ammonium hydroxide and mixtures thereof. The slurry can be made acidic using any suitable acid which provides the desired acidity and does not unduly interfere with the methods and products of the present invention. Examples of such acids include acetic acid, other carboxylic acids and mixtures thereof.

In the present collagen-containing composites, the weight ratio of total calcium, phosphate-containing component to the amount of collagen component is preferably in the range of about 0.03 to about 2.5.

The present collagen-containing composites are different from previous collagen-calcium phosphate ceramic composite materials. The later are simply physical mixtures with collagen as binder for the calcium phosphate ceramics. No interaction between the collagen and the calcium phosphate ceramics occurs. In contrast, the present composites contain a calcium, phosphate-containing component which is incorporated into the structure of the collagen component, and is preferably interacted with the collagen component. The present composites are substantially homogeneous, preferably with little or no phase separation being visually apparent. The present composites have substantial compositional flexibility and the weight ratio of precipitated calcium, phosphate-containing component to collagen component can be varied over a relatively wide range. By doing this, the mechanical strength and/or bioreabsorption rate of the present composites can be varied over relatively wide ranges. In addition, the present composites, which contain precipitated calcium apatite incorporated into collagen structure, have properties which are similar to the properties of natural bone.

It should be noted that in this invention the order of precipitation is quite important. For example, if calcium apatite is formed in the collagen free solution, the addition of this calcium apatite into a collagen slurry still shows two phases of materials including collagen and a separate phase of calcium apatite. This is different from the present substantially homogeneous composite materials.

The hydroxyapatite ceramics, other apatite ceramics and calcium, phosphate-containing bioglasses are normally prepared by sintering techniques at high temperatures. In one embodiment of the present invention, a substrate, preferably made from a material selected from calcium apatite ceramics, calcium, phosphate-containing bioglasses and mixtures thereof, is contacted with, preferably immersed or soaked in, an oversaturated or supersaturated solution, preferably an aqueous solution, of a coating material selected from calcium, phosphate-containing components, preferably selected from calcium apatites and mixtures thereof, preferably at a pH of about 6.5 or about 7.0 or higher. This contacting results in a coating of precipitated material selected from calcium, phosphate-containing components, preferably selected from calcium apatites and mixtures thereof, being deposited on at least a portion of the external surfaces of the substrates.

Suitable such oversaturated solutions can be prepared by mixing a calcium ion-containing solution and a phosphate-containing ion-containing solution. The total calcium concentration of such oversaturated solutions is preferably in the range of about 1.0×10^{-3} M to about 3.0×10^{-3} M and the total phosphate concentration of such oversaturated solutions is preferably in the range of about 1.0×10^{-3} to about 2.0×10^{-3} M.

Suitable calcium salts for the preparation of such oversaturated solutions are, for example, soluble calcium salts, such as calcium chloride, calcium nitrate, calcium acetate, other soluble calcium carboxylates, other soluble calcium organic salts and mixtures thereof. Suitable phosphate salts for the preparation of such oversaturated solutions are alkali metal phosphate salts, ammonium phosphate salts and mixtures thereof. Specific examples include monobasic alkali metal phosphates, dibasic alkali metal phosphates, tribasic alkali metal phosphates and mixtures thereof. pH adjusting

agents such as alkali metal hydroxides, ammonium hydroxide and mixtures thereof may be employed.

The solubility of calcium apatites and other calcium phosphate salts is very sensitive to the pH of the liquid medium. In general, solubility of calcium apatites and other calcium phosphate salts decreases with increasing pH. The metastable limit of a calcium apatite oversaturated solution also decreases with increasing pH. In principle, any oversaturated solution can be used for contacting the present substrates to induce the precipitation of one or more calcium, phosphate-containing components, e.g., calcium apatites, on the substrate's surface. However, it is preferred to employ a stable metastable solution. By contacting the substrate with such a solution, the precipitated calcium, phosphate-containing component is deposited preferentially on the surfaces of the substrate rather than in the bulk of the solution. If the solution is excessively oversaturated, the solution is very unstable and bulk precipitation occurs immediately. In this case, the solution concentration decreases rapidly to the normal or saturated solubility values and the chances of deposition on the substrate surface are reduced.

The oversaturated solutions can be prepared by mixing required amounts of calcium ion- and phosphate ion-containing solutions at low pH (pH < 6.0). At this low pH, the combined solution is undersaturated. The solution pH is then raised, preferably to 6.5 or about 7.0 or higher, for example, with diluted alkaline solution, until it reaches the metastable oversaturated region. The substrate can then be contacted with such solution. This contacting step can be repeated several times by using a different, for example, newly prepared, oversaturated solution for each contacting until the external surfaces of the substrate are covered by the desired amount of deposited precipitated calcium, phosphate-containing component. Other soaking methods which may be employed include the continuous flow of a metastable oversaturated solution to a reactor tank which contains the substrate for treatment. The contacting or contactings preferably continue until the newly formed precipitated material is attached to the surface of the substrate. The contacting solution is preferably maintained in the oversaturated condition during the treatment.

Because the precipitated calcium, phosphate-containing component, e.g., one or more calcium apatites, is more close to the biological apatite than the sintered calcium apatite ceramics or calcium, phosphate-containing bioglasses, the present coated substrates interact and bond with bone more quickly than do the uncoated substrates. Therefore, the healing time is shortened and the ceramic-bone bonding strength is stronger using the present coated substrates relative to the uncoated substrates.

The oversaturated solutions may contain some fluoride ion which induces the deposition of fluorapatite instead of hydroxyapatite. In such cases, the fluoride ion is preferably contained in the phosphate-containing solution before mixing with the calcium-containing solution.

Pure hydroxyapatite ceramic has a surface pH normally ranging from about 7.0 to about 10.0. In order to accelerate the formation of precipitated calcium apatite or fluorapatite on the surface of such ceramics, the ceramics can be prepared to include about 0.5% to about 3% unreacted calcium oxide. Ceramics which include excess calcium oxide normally have a surface

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higher. By introducing this ceramic
ution, the unreacted lime leaches out
um ions and high surface pH, which
ditions for the deposition of precipi-
ites. In such case, the formation of
m apatites on the surfaces of the sub-
on substrates made of pure hydroxy-
The oversaturated solution for treat-
ame as before. However, in certain
the leachable calcium ion is high
on for treatment can only have phos-
phate ion with fluoride ion.
non-limiting Examples illustrate cer-
of the present invention.

EXAMPLE 1

agen (type 1 collagen obtained from
n) was added into 400 ml water and
al acetic acid was then added to this
nd stirred. At this point, the slurry
of a homogeneous gel. 4.4 g CaCl₂
as then dissolved into the collagen
mixture was adjusted to higher than
OH.

was separately dissolved in 30 ml of
phosphate solution was added
ove-noted collagen-containing slurry
mixed vigorously in the blender.

en filtered and the recovered solid
ed several times with deionized wa-
lid material was shaped and dried in

e dried material was visually exam-
ow separate phases of collagen and
s. This dried material, which was
eight collagen and about 67% by
atite, became very hard and did not
lling in water as did pure collagen.
rial is useful as a hard tissue, e.g.,
material.

EXAMPLE 2

ype 1 collagen derived from ReGen
dispersed in 200 ml water containing
etic acid to produce a slurry in the
ous gel.

as dissolved in 10 ml of water. This
d to the above-noted slurry and
the resulting slurry was adjusted
gher than 10.

4 was dissolved in 15 ml of water.
solution was added to the above-
e slurry was strongly mixed in a

tered, and the recovered solid mate-
veral times with deionized water.
material was collected and shaped,

ite, which included about 67% by
33% by weight calcium phosphate,
us. No separate phases of collagen
ate were observed This dried mate-
ard tissue, e.g., bone, replacement

on has been described with respect
amples and embodiments, it is to be
invention is not limited thereto and

that it can be variously practiced within the scope of the
following claims.

What is claimed is:

1. A composition useful as a hard tissue replacement
material comprising a synthetically derived, substan-
tially homogenous, solid composite consisting essen-
tially of about 25% to about 95% by weight of collagen
component and about 5% to about 75% by weight of
calcium, phosphate-containing component precipitated
from an aqueous liquid medium in which said collagen
component and a soluble calcium-containing compo-
nent and a soluble phosphate-containing component
from which said calcium, phosphate-containing compo-
nent is derived are contained, said aqueous liquid me-
dium prior to said precipitation being free of insoluble
calcium-component and insoluble phosphate containing
component.

2. The composition of claim 1 wherein said collagen
component is type 1 collagen.

3. The composition of claim 1 wherein said calcium,
phosphate-containing component is selected from the
group consisting of calcium phosphates, calcium apa-
tites, and mixtures thereof.

4. The composition of claim 1 wherein the mole ratio
of calcium to phosphate is in the range of about 1 to 2.

5. A method for producing a collagen-calcium, phos-
phate-containing substantially homogenous solid com-
posite comprising:

forming a mixture containing aqueous liquid, colla-
gen, and either a soluble calcium-containing com-
ponent or a soluble phosphate-containing compo-
nent;

combining said mixture with an aqueous liquid con-
taining said soluble calcium-containing component
or said soluble phosphate-containing component
which is not present in said mixture, and subjecting
said combination to conditions effective to cause
the precipitation of a calcium, phosphate-contain-
ing component derived from said soluble calcium-
containing component and said soluble phosphate-
containing component, said combination prior to
said precipitation being free of insoluble calcium-
containing component and insoluble phosphate
containing component; and

recovering from said collagen and said precipitated
calcium, phosphate-containing component a solid
collagen-calcium, phosphate-containing material
consisting essentially of about 25% to about 95%
by weight of collagen and about 5% to about 75%
by weight of said calcium, phosphate-containing
component.

6. The method of claim 5 wherein said combination is
agitated while being subjected to said conditions.

7. The method of claim 5 wherein said conditions
include a pH of at least about 7.

8. The method of claim 5 wherein said conditions
include a pH of at least about 10.

9. The method of claim 1 wherein said solid collagen-
calcium, phosphate-containing material is a substan-
tially homogenous solid composite.

10. The method of claim 9 wherein said recovering
step comprises separating said solid collagen-calcium
phosphate-containing material from one or more com-
ponents other than collagen and said precipitated cal-
cium, phosphate-containing component present with
said collagen-calcium, phosphate-containing material.

11. The method of claim 10 wherein said recovering
step further comprises drying said solid collagen-cal-
cium, phosphate-containing material.

12. A method for producing a collagen-calcium,
phosphate homogeneous solid composite comprising:
forming a mixture containing an aqueous liquid and
collagen;

simultaneously combining said mixture with a first
aqueous liquid containing a soluble calcium-con-
taining component and with a second aqueous liq-
uid containing a soluble phosphate-containing
component at conditions effective to cause the
precipitation of a calcium, phosphate-containing
component derived from said soluble calcium-con-
taining component and said soluble phosphate-con-
taining component, provided that said mixture, said
first aqueous liquid and said second aqueous liquid
prior to said precipitation are free of insoluble cal-
cium-are free of insoluble calcium-containing com-
ponent and insoluble phosphate-containing compo-
nent; and

recovering from said collagen and said precipitated
calcium, phosphate-containing component a solid
collagen-calcium, phosphate-containing material

consisting essentially of about 25% to about 95%
by weight of collagen and about 5% to about 75%
by weight of said calcium, phosphate-containing
component.

13. The method of claim 12 wherein said first aqueous
liquid and said second aqueous liquid each have a pH of
at least about 7.

14. The method of claim 12 wherein said first aqueous
liquid and said second aqueous liquid each have a pH of
at least about 10.

15. The method of claim 12 wherein said solid colla-
gen-calcium, phosphate-containing material is a sub-
stantially homogenous solid composite.

16. The method of claim 15 wherein said recovering
step comprises separating said solid collagen-calcium,
phosphate-containing material from one or more com-
ponents other than collagen and said precipitated cal-
cium, phosphate-containing component present with
said collagen-calcium, phosphate-containing material.

17. The method of claim 16 wherein said recovering
step further comprises drying said solid collagen-cal-
cium, phosphate-containing material.

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