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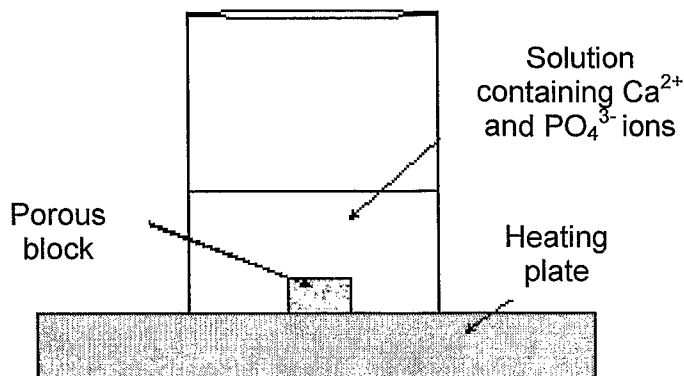
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TAINED POROUS PRODUCT



(57) Abstract: The present invention describes a process for coating an interconnected porous substrate, synthesis intermediates and interconnected porous coated products. The process of the present invention consists of the deposition, on porous blocks (*scaffolds*), with mineral materials, optionally converted into other materials. The products of the present invention are useful in several applications, emphasizing applications for bone regeneration and tissue engineering and in molecules delivery or uptake.

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### Descriptive Report

#### PROCESS FOR POROUS INTERCONNECTED SUBSTRATE COATING, SYNTHESIS INTERMEDIATE AND OBTAINED POROUS PRODUCT

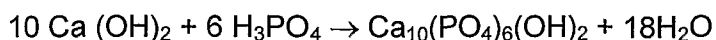
##### 5 Invention Field

The present invention is related to a process for coating an interconnected porous substrate, to synthesis intermediates and to coated porous interconnected products. More specifically, the process of the present invention consists of inorganic compounds deposition on porous blocks (scaffolds). The inorganic compounds, such as calcium phosphates and other salts, can be optionally converted into other compounds. The products of the present invention are useful in several applications, especially applications for bone regeneration, tissue engineering and drug or other molecules delivery or uptake.

15

##### Invention Background

Solid and porous substrates coating process through mineral salts deposition are known. One of the known methods consists of coating metallic devices in a bath, electro deposition, among others. As an example, one can mention titanium devices coating by electrophoresis, where hydroxyapatite is precipitated by ammonia addition to a solution rich in  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions. In this method, a solution with the following composition is used: 0,5M  $\text{Ca}(\text{OH})_2$ , 0,3M  $\text{H}_3\text{PO}_4$  e 1M  $\text{CH}_3\text{CHCO}_2\text{HOH}$  (lactic acid). The referred solution is transparent and has a pH value of 3.76. Under these conditions, hydroxyapatite precipitation is caused by ammonia addition, so that the solution pH value reaches the range of thermodynamic stability for hydroxyapatite. Hydroxyapatite precipitation occurs according to the reaction indicated below:

25  
30

The above described process produces a pure hydroxyapatite coating. However, the coating is poorly adhered to the substrate. Additionally, such

process does not coat porous substrate so that the mineral coating acquires the shape of the used substrate. Such characteristics are strongly desired in several practical situations, being highlighted herein, just to illustrate but not to limit the cases in which such structures are used in the general field of biosciences. This kind of structure (porous material) has been intensively studied due to its potential applications in the fields of bone regeneration and bone engineering, and several other fields. In this technical field, autographs (bone from the same patient) and allograft (bone from other individual from the same specie) are the most widely used for inducing bone regeneration. Such processes consist, generally, of the introduction of bone pieces in the place of injury so that it is filled with newly-formed bone. It is worth to mention that there is a critical size above which the human body cannot heal itself, being necessary to use grafts. When the size of the defect is too large, it comes to be necessary the introduction of a large quantity of bone pieces to foster regeneration. In some cases, a porous block is necessary in order to provide an immediate structural support. Autologous graft is mostly used to foster regeneration. However, this procedure requires an additional surgery to remove bone from other area in the patient's body, generally from the iliac. However, it is well known that approximately 30% of the patients submitted to this kind of surgery suffer with strong pain during a long time, being this pain several times more intense than the pain that the surgery procedure intended to correct. In addition to the uncomfortable situation, there is the limitation concerning the amount of bone possible to be retrieved from the iliac. The formation of a defect bigger than the critical size would generate the necessity of a new graft in the region from where the bone pieces were retrieved.

An alternative to the autologous and allogeneous grafts is the use of alloplastic grafts to foster bone regeneration. Examples of these agents include biomaterials constituted of some calcium phosphates, such as hydroxyapatite, tricalcium phosphate and fluorapatite, once they are applied as biocompatible minerals due to being the main constituents of hard tissues. Hydroxyapatite and

its modified variations, is the object of a great interest and importance due to the fact of being a compound present in bones, cartilage and dentine.

Bone can be injured by fracture or large defects can be caused by trauma or pathology such as tumors; bone can be weakened by osteoporosis or  
5 by drug side effect. In such situations, bone defect must be filled. There are some restrictions concerning the nature, structure and composition of the material to be used as bone substitute. This material must be physiologically compatible in order to avoid the formation of coagulum, inflammatory response and so on. In addition, there must be a strong integration between the material  
10 and the newly-formed bone. It is also desirable that the material assume some natural functions of bone such as: hosting undifferentiated cells, allow remodeling by osteoclasts followed by new bone growth and calcium metabolic exchange.

During the treatment of this kind of patient as well as patients with health  
15 conditions requiring administration of one or more medicine in pre-determined doses, it is essential that the intended medical administration be effectively fulfilled. One way of searching a better control of the effective drug administration is the use of controlled drug delivery systems. Although several technologies related to controlled drug delivery are well known such as those  
20 described in the patent US 7,001,371, the present inventors do not know the use of porous interconnected coated with mineral materials as a strategy for controlled drug delivery systems.

Patent literature encompasses several patents related to mineral formation from other materials in order to obtain products with desired shape.  
25 As an example, the American patent US 4,880,610 describes the production of calcium phosphate minerals through the reaction of concentrated phosphoric acid with an alkaline-earth metal source, particularly calcium, in the presence of an alkali and hydroxyapatite crystals. The components are intensively mixed and the product is shaped.

Patent literature also encompasses several examples of patents relate to the production of porous blocks for bone regeneration and tissue engineering. Some of them are cited herein as a matter of reference.

The Brazilian patent PI 8201050-1 describes the process of production of titanium dioxide concentrate, metallic iron and other sub-products from ilmenite and similar minerals. The process consists of executing a selective reduction in the mine, being or not proceeded by oxidation followed by lixiviation of the reduced material and finally submitting the solution to an electrodeposition process.

The North American patent US 6,953,594 is related to a bioactive ceramic material containing calcium phosphate and apatite crystals with Ca/P ratio compared to that naturally occurs in bone, and also with hardness similar to that of bone. This ceramic material is produced at low temperature, biocompatible and bio-resorbable.

The North American patent US 6,899,873 is related to methods of fabrication of micro tubular polymeric porous materials. These methods are related to the production of porous biodegradable polymeric blocks, to be used in tissue regeneration, nervous channels regeneration, medical devices (artificial kidney, dialysis devices), porous channels, catalytic substrates, among others. In this method, the mixture solution and/or emulsion is prepared by mixing the polymer(s) to the solvent(s) with or without stirring and with or without heating. Temperature is, then, altered in order to cause phase separation and the residual phase is removed.

The North American patent US 7,001,371 describes methods and an apparatus to control cellular growth in porous implants produced with low-density ceramic. These porous blocks act as reservoirs for drug transport, mainly in vascular tissues.

However, there is still a demand yet not supplied by process capable of, simultaneously: (i) provide adhesion to a substrate; (ii) acquire the substrate shape over which the coating is produced; (iii) cause the primary substrate removal so that the coated material gets its shape and maintains it even after

the primary substrate removal; (iv) cause chemical modification of the coating layer without altering substantially the shape obtained by the coating; (v) cause the formation of synthesis intermediates for the production of interconnected porous structures, structures those containing materials to which there are not obtainance processes that result specifically in the desired porous structures or that such processes are disadvantageous. The present invention provides such processes and products, overcoming a gap in this technical field.

### **Invention Abstract**

One of the objects of the present invention is to provide a process for coating a porous interconnected substrate, the referred process consisting of the deposition of mineral materials on porous blocks (scaffolds).

In one aspect, the coating process of the present invention allows the adhesion of the mineral material to the substrate so that the mineral coating gets the substrate shape on which the coating is done. It is, thus, another object of the present invention to provide a process for obtaining mineral materials with varied shapes, depending on the utilized substrate. It is also an object of the present invention the porous interconnected product coated with mineral material.

In another aspect, the coating process of the present invention allows the removal of the primary substrate, so that the coated material acquires its shape and maintains it even after the primary substrate removal. It is, thus, another object of the present invention to provide a process for obtaining mineral materials with varied shapes, depending on the utilized substrate, in which the substrate is removed without damage to the structure acquired by the coating. It is also an object of the present invention the interconnected porous product of mineral material.

In still another aspect, the coating process of the present invention allows the chemical modification of the coating layer without substantially altering the shape obtained by the coating. It is, thus, still another object of the present invention to provide a process for obtaining mineral materials with variable

shapes and chemical composition, depending on the architecture of the substrate and on the preparation conditions utilized, in which the coating is chemically modified without substantial loss of the shape obtained by the coating. It is also an object of the present invention the interconnected porous  
5 product of chemically modified mineral material.

In another aspect, the interconnected porous structures coated with mineral material of the invention (optionally modified) are useful as synthesis intermediate of other materials, maintaining the porous structure of the referred intermediate and acquiring other functionalities/applications consequent from  
10 the type of material used on such intermediates. Such intermediates are particularly useful in the cases where there are not obtaining processes that specifically result in the desired porous structures for certain materials or that such processes are disadvantageous. It is, thus, another product of the present invention to provide synthesis intermediates of other interconnected porous  
15 materials.

In a preferential aspect, the present invention provides a process of production of porous blocks coated with hydroxyapatite, from the hydrothermal deposition of monetite on a porous substrate and further monetite conversion to pure hydroxyapatite. This material is useful as block, mould or scaffold for bone  
20 regeneration and/or tissue engineering, being this material, thus, still another object of the present invention.

In another preferential aspect, the present invention provides a process of production of coated porous blocks that perform the function of molecules transport by diffusion. This material is useful in molecules delivery systems such  
25 as drugs (in which case the gradual delivery can be obtained) and other substances, being the use of this material for such aims, thus, still another object of the present invention.

In another preferential aspect, the present invention provides a process of production of coated porous blocks that perform the function of molecules  
30 uptake by diffusion. In this case, the polymeric substrate is not necessarily eliminated. There is a porous block composed by polymer coated with calcium

phosphate. This material is useful in environmental applications such as filters for heavy metal uptake and separation processes, among other applications, being the use of this material for this aim, thus, still another object of the present invention.

5 In still another preferential aspect, the present invention provides a process of production of porous blocks composed by biocompatible polymer coated with hydroxyapatite that perform the function of scaffolds for bone regeneration. In this case, the polymeric substrate is not necessarily eliminated; being the use of these materials for such aims, thus, still another object of the  
10 present invention.

These and other objects of the present invention will be better understood and valued from the detailed description of the invention and of the attached claims.

#### 15 **Brief Description of Figures**

Figure 1 shows a schematic representation of a bath for hydrothermal deposition of the ceramic material on the interconnected porous substrate, where indicated: the solution containing the ions  $\text{Ca}^{2+}$  e  $\text{PO}_4^{3-}$ ; the porous substrate to be coated.

20 Figure 2 illustrates some examples of the porous products of the present invention, in which 2a corresponds to a scanning electron microscopy (SEM) image of the sponge coated with monetite, not sintered; 2b corresponds to an SEM image of the porous sintered, biphasic (hydroxyapatite + tricalcium phosphate) ceramic block; 2c corresponds to an optical microscopy image of a  
25 biphasic porous block. Figure 2d corresponds to different products obtained from different interconnected porous substrates.

Figure 3 is a scanning electron microscopy (SEM) micrograph of a porous product coated by the process of the present invention.

30 Figure 4 is a scanning electron microscopy (SEM) micrograph of a coated and chemically modified porous product by the process of the present invention.



Figure 5 shows the X-ray diffraction analysis results in metallic niobium samples coated with monetite by the same coating method utilized in the present invention. The samples were analyzed in synchrotron radiation. In the time point  $t=5$  minutes, the coating is composed by pure monetite while at  $t = 30$  minutes, peaks corresponding to intermediate phases are identified.

Figure 6 shows the X-ray diffraction analysis results in metallic niobium samples coated with monetite and immersed in NaOH solution with  $\text{pH}=12.5$ , at different time points:  $t = 1, 4$  and 24 hours.

Figure 7 shows the scanning electron microscopy analysis results in samples immersed in KOH or  $\text{NH}_4\text{OH}$  solutions with  $\text{pH}=12.5$ , during 48 (forty eight) hours.

Figure 8 shows SEM images indication scaffolds bioactivity after incubation in SBF during 24 hours. [

Figure 9 shows SEM images of bone marrow cells cultured during 48 hours on biphasic scaffolds.

Figure 10 shows SEM images of adipose tissue cells cultured during 48 hours on biphasic scaffolds.

### **Detailed Description of the Invention**

The inventors, in the search for an alternative solution to the presented problem, developed a process interconnected porous substrates coating, as well as modifications of this process and also developed new products.

An improved process for coating an interconnected porous substrate is described as follows, which consists of the deposition of mineral materials on porous blocks (scaffolds) with variable dimensions, being produced from the process of the present invention microporous or macroporous scaffolds, or, since some few micrometers to several millimeters. In the case of microporous scaffolds, scaffolds with pore diameters ranging from 20-50 can be produced. The minimum pore size occurs in the limiting condition in which the substrate pores on which the coating is performed close by the coating thickening of adjacent interconnections. One may observe that, to this happen, there would

be necessary successive coatings on a substrate with pores, for example, in the range of 300 micrometer of diameter. In the case of a "more closed" porous substrate, or, with pore diameters of nearly 50 micrometers, there would be necessary less repeated coatings. In this case, micropores are formed (or  
5 mesopores), which will have final dimensions according to the sintering temperature and to the thermal cycle.

The coating process of the present invention provides the adhesion of the mineral material to the substrate, so that the mineral coating acquires the shape of the substrate on which the coating is done. For this reason, it is a  
10 process for obtaining mineral materials with variable shapes, depending of the utilized substrate shape, resulting in an interconnected porous product coated with mineral material. For purposes of the present invention, the word "mineral" means any mineral selected from the group encompassing monetite ( $\text{CaHPO}_4$ ), brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), alumina ( $\text{Al}_2\text{O}_3$ )  
15 and zirconia ( $\text{ZrO}_2$ ) or yet combinations of them. In these conditions, an interconnected porous substrate is submitted to coating at controlled temperature, during certain time period, in solution rich in calcium and phosphate ions and in solution containing the same constituents and different metals. This process provides the deposition of positive ions on the negatively  
20 charged substrate surface that, for its time, foster the bonding of negatively charged ions, in a manner to nucleate crystals of mineral material with different chemical compositions and stoichiometries on the substrate surface. The substrates and ions solutions are chosen according to the kind of coating desired, as well as concentration, temperature and time conditions. The  
25 procedure can be repeated several times in order to obtain desired coating thicknesses. After deposition, the coated substrate can be optionally washed with distilled water and air dried. The coated product can be characterized by several techniques such as X-ray diffraction and scanning electron microscopy (SEM).

30 In another concretization of the present invention, the process of coating an interconnected porous substrate includes the removal of the primary

substrate so that the coated material acquires its shape and maintains it even after removal of the primary substrate, without damage to the structure acquired by the coating. The primary substrate removal can be performed after the coating process described above or after the application of processes of coating  
5 modification. The interconnected porous substrate removal that serves as scaffold for the coating is performed through the chemical or physical dissolution or destruction of the substrate, maintaining the integrity of the coating by the use of conditions in which the coating is neither degraded nor dissolved.

10 In another concretization of the present invention, a chemical modification process of the coating, without substantially altering the shape obtained by the coating, providing, thus, an interconnected porous product of chemically modified mineral material is also provided. The chemical modification of the coating is obtained by the immersion in a solution containing  
15 other ions and/or chemical substances capable of reacting with the coating material, or, materials capable of react with minerals.

The interconnected porous structures coated with mineral materials of the invention (optionally modified) are useful as synthesis intermediates of other materials, maintaining the porous structure of the referred intermediate and  
20 acquiring other functionalities/applications from the type of material used on such intermediates. Such intermediates are particularly useful in cases where there are not processes of obtaining that specifically result in the desired porous structures for certain materials or that in such processes are disadvantageous.

25 In a preferential concretization of the present invention, a process of production of porous blocks coated with stoichiometric and calcium deficient hydroxyapatite is provided, as well as hydroxyapatite doped with metals and anionic groups, from the hydrothermal deposition of monetite on a porous substrate and further monetite conversion to pure hydroxyapatite. This material  
30 is useful as block, mould or scaffold for bone regeneration and/or tissue engineering. This concretization of the present invention presents, as

advantage, the fact that the conversion can be done with other alkali solution such as potassium hydroxide (KOH) and ammonium hydroxide, being both solutions preferentially at 60°C and with pH = 12.5, being this the same as that of a solution 0.1M NaOH. The structure so generated consists of a porous product with interconnected porosity and pore diameters higher than 100µm, being this requirement essential to allow bone ingrowth, for example. It is worth to mention that the shape and dimensions of the pores are a function of shape and dimensions of the utilized sponge, as well as the number of coatings.

In another preferential concretization of the present invention, a product is provided, that allows a better control of the effective drug administration intended by the use of controlled drug delivery systems. Although several technologies related to controlled drug delivery are well known, the present inventors do not know the use of porous interconnected coated with mineral materials as a strategy for controlled drug delivery systems. For this reason, the process of production of coated porous blocks also performs the function of molecules transport by diffusion, being this material also useful in systems of gradual drug or other substances delivery.

The present invention also provides a process of production of coated porous blocks that perform the action of uptake or delivery of molecules by diffusion. This material is useful in environmental applications such as filters, in the uptake of heavy metal and in separation processes among other applications.

An industrial process presents yet as an advantage, the fact that the use of relatively low temperatures involves an economy of energetic waste, presenting a more economic process in comparison to commercial processes.

The following examples illustrate, but not limit, some of the ways of performing different objects of the present invention.

#### **Example 1 – Interconnected porous substrate coating with mineral material**

In this preferential concretization, the coating process of the present invention allows the adhesion of a mineral material to the polymeric substrate, so that the

mineral coating acquires the shape of the substrate on which the coating is done. In this example, a process of depositing monocalcium phosphate ( $\text{CaHPO}_4$  - monetite) on polymeric sponges is described. Polymeric sponges, chosen according to their pores architecture, are immersed in a recipient (Figure 1) with an aqueous solution rich in ions calcium and phosphate, at  $80^\circ\text{C}$ , during one hour. After this time,  $\text{Ca}^{2+}$  are deposited on the negatively charged surface of the sponges and bond to  $\text{PO}_4^{3-}$  ions, nucleating monetite  $\text{CaHPO}_4$ , crystals. This procedure can be repeated several times in order to obtain desired coating thicknesses. Figure 2 shows several examples of products obtained by the above process. Figure 3 shows a product of the process above described, in which the coating is characterized by scanning electron microscopy (SEM). Figure 5a shows a result of characterization of the product from the process above described, in which the coating is characterized by X-ray diffraction. One can observe a continuous layer in Figure 3 (SEM), composed of monetite parallelepiped-like crystals of approximately  $8 \times 4 \times 2 \mu\text{m}$ . This layer is characterized as pure and crystalline monetite by X-ray diffraction (Figure 5a).

**Example 2 – Chemical modification of the interconnected porous product coated with mineral material**

In this preferential concretization, the process for chemical modification of the coating of the present invention provides a chemical modification of the coating layer, without substantially altering the shape obtained by the coating, providing, thus, a chemically modified interconnected product of mineral material. Taking as an example the product obtained in the previous example, a pure monetite interconnected porous block, its chemical modification is obtained by the sample immersion in a  $\text{NaOH}$  ( $\text{pH}=12.5$ ) solution at  $60^\circ\text{C}$ , during 24 hours. This process provides the hydrothermal conversion of monetite to hydroxyapatite, giving rise to a hydroxyapatite interconnected porous product. The sample can then be washed in ultra-pure water for removing the remaining solution, dried at  $60^\circ\text{C}$  overnight. The observation of this product in a

microscope (Figure 4) reveals a significant morphological change in the micrometric scale. It is worth to highlight that the shape, at macroscopic scale, is maintained. Nanocrystals precipitation is observed, visible with magnification of 75000x, on the previous parallelepiped-like crystals of monetite. X-ray diffraction analyses (Figure 6c) reveal that the coating, after conversion, consist  
5 of pure and crystalline hydroxyapatite.

### **Example 3 – Primary substrate removal**

In this preferential concretization, the process of the present invention  
10 provides the removal of the primary substrate, on which the coating layer was deposited, without substantially altering the shape obtained by the coating or its modification. Taking as an example the product of example 1 or the product of example 2, one proceeds to the primary substrate removal (in these cases, polymeric sponges) by thermal treatment in two steps. In the first one, the  
15 samples are heated at 550°C at low heating rates, between 0.5°C/min. and 1°C/min.

**Example 4 – Interconnected porous product coated with mineral material and chemically modified in different process conditions.**

20 Different process conditions were tested for example 2, of interconnected porous product production process, coated and chemically modified. In these tests, the evolution of the conversion from monetite to hydroxyapatite was assessed by SEM observation and X-ray diffraction in samples immersed in NaOH solution with pH=12.5, in different time points:  $t = 0$ , 5 and 15 minutes;  
25 and  $t = 1$ , 4, 8, 24 and 48 hours. As it can be observed in Figures 6 and 7, X-ray diffraction and SEM analyses revealed that the time of 4 (four) hours is enough for the complete conversion from monetite to hydroxyapatite to occur.

**Example 5 – Interconnected porous product coated with mineral material and chemically modified – lattice parameters analysis.**  
30

Applications of Interconnected porous product, coated and chemically modified, depend on its microstructure. To assess the applicability of the of the present invention products to biomedical applications, lattice parameters of the interconnected porous products coated and modified to hydroxyapatite from example 2 or from example 4 were measured, that is, obtained in the different time points, using to do so, the technique of X-ray diffraction with Rietveld structural refinement. The results from Table 1 show that there was no significant change on hydroxyapatite lattice parameters. However, Fourier transform infra-red spectroscopy results indicated the substitution of carbonate groups in the structure of hydroxyapatite. This result indicates that the obtained material is more similar to bone apatite than to pure and crystalline synthetic hydroxyapatite.

**Table 1.** Lattice parameter data for the invention products.

TIME(h)	a (Å)	c (Å)	a/c
THEORETICAL	9.4180	6.8840	1.368
1	9.3945	6.8696	1.367
4	9.4049	6.8820	1.367
8	9.3967	6.8910	1.364
24	9.4079	6.9029	1.363
48	9.4180	6.8900	1.367

**Example 6** – Chemical modification of the interconnected porous product coated with mineral material – other chemical modification conditions

In this other preferential concretization, the process for chemical modification of the present invention coating provides yet other conditions for chemical modification of the coating layer, without substantially altering the shape obtained by the coating, providing, thus, an interconnected porous product of chemically modified mineral material. Taking as an example the product obtained in example 1, a pure monetite interconnected porous block, its chemical modification is obtained by immersion of the coated sample in a KOH

or  $\text{NH}_4\text{OH}$  ( $\text{pH}=12.5$ ), at  $60^\circ\text{C}$ , during 24 hours. The sample can then be washed in ultra-pure water in order to remove the remaining solution and dried at  $60^\circ\text{C}$  overnight. Figure 7 presents the microstructure of a hydroxyapatite sample, obtained by the conversion with  $\text{KOH}$ .

5

**Example 7** – Use of the interconnected porous product coated with mineral material and chemically modified in the preparation of a product for bone regeneration and/or tissue engineering

In this preferential concretization, a product prepared in the conditions illustrated in the examples 4 and 6 is used in the preparation of product for bone regeneration and/or tissue engineering. Bioactivity tests were performed in simulated body fluid (SBF). Figure 8 confirms the spontaneous precipitation of bone-like apatite after seven days in SBF. Literature shows that this precipitation only occurs in those bioactive materials, or, able to interact with body fluids fostering this spontaneous precipitation, fact indicative that the material will establish chemical bond with newly-formed bone tissue. The material was tested with cells from adipose tissue, Figure 9, and bone marrow cells, Figure 10. The results indicated that the material is biocompatible, as the cells adhered to the substrates and spread on the material. It was observed yet, intense cell proliferation.

The ones versed in art will value the knowledge herein presented and will be able to reproduce the invention in the modalities presented and in other variations, encompassed in the scope of the attached claims.



### Claims

#### PROCESS FOR POROUS INTERCONNECTED SUBSTRATE COATING, SYNTHESIS INTERMEDIATE AND OBTAINED POROUS PRODUCT

- 5 1. Process for coating an interconnected porous substrate, characterized by contains a hydrothermal coating step with a mineral material on the referred substrate, this mineral material being selected from the group that encompasses monetite ( $\text{CaHPO}_4$ ), brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), alumina ( $\text{Al}_2\text{O}_3$ ) and zirconia ( $\text{ZrO}_2$ ).
- 10 2. Process, as in claim 1, characterized by the fact that the coating step includes, apart from the referred constituents, solutions containing metallic ions, fluorine, chlorine and anionic groups in order to produce coatings with impurities from the referred elements.
- 15 3. Process, according to claims 1 or 2, characterized by the fact that the referred coating is carried out at temperatures in the range of 20°C and 150°C.
- 20 4. Process, according to claims 1 or 2, characterized by the fact that the coating time is adjusted according to the desired coating thickness.
5. Process according to claims 1 or 2, characterized by the fact that the referred coating is carried out at temperatures in the range of 60°C and 100°C.
- 25 6. Process, according to any previous claim, characterized by containing an additional step of chemical modification of the referred coating.
- 30 7. Process, according to claims 1 or 2, characterized by the fact that the coating is composed by monetite or brushite and the chemical modification converts the coating to stoichiometric hydroxyapatite, calcium deficient hydroxyapatite and hydroxyapatite containing metallic and anionic impurities.

8. Process, according to any previous claim, characterized by additionally dipping the already coated polymeric substrate in an alkali solution, under heating.

5

9. Porous product of interconnected mineral material characterized by the fact of containing the three-dimensional substrate structure on which the referred product is deposited.

10 10. Product, according to claim 9, characterized by the fact that the referred substrate is optionally removed from the structure.

11. Product, according to claims 9 or 10, characterized by the fact of being chemically modified.

15

12. Product, according to claims 9 to 11, characterized by the fact the coating is composed by monetite or brushite and the chemical modification converts the coating to hydroxyapatite.

20 13. Product, according to claims 9 to 12, characterized by enclosing interconnected micropores with dimensions between  $1\mu\text{m}$  and  $1000\mu\text{m}$ .

14. Product, according to claims 9 to 12, characterized by enclosing interconnected macropores with dimensions between 1mm and 100mm.

25

15. Use, in the preparation of product for bone regeneration and/or tissue engineering, of porous product of interconnected mineral material, optionally chemically modified, characterized by the fact that the referred porous product encloses the substrate three-dimensional structure on which the referred product is deposited.

30

16. Use, in the preparation of a product for delivery and/or molecule uptake, of porous product of interconnected mineral material, optionally chemically modified, characterized by the fact that the referred porous product encloses the substrate three-dimensional structure on which the referred product is deposited.

17. Synthesis intermediate, characterized by enclosing porous product of interconnected mineral material, obtained by hydrothermal coating of a porous substrate with a mineral selected from the group containing monetite ( $\text{CaHPO}_4$ ), brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), tricalcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), alumina ( $\text{Al}_2\text{O}_3$ ) and zirconia ( $\text{ZrO}_2$ ).

18. Synthesis intermediate, according to claims 17, characterized by the fact that the referred porous product additionally encompasses chemical modification.

19. Synthesis intermediate, according to claims 18, characterized by the fact of enclosing hydroxyapatite-based bioceramics, with porous and interconnected structure.

**AMENDED CLAIMS**

[Received by the International Bureau on 18 OCT 2007 (18.10.2007)]

**Amended Claims**

PROCESS FOR POROUS INTERCONNECTED SUBSTRATE COATING,  
SYNTHESIS INTERMEDIATE AND OBTAINED POROUS PRODUCT

1. Process for coating of a porous substrate **characterized by** the fact that the coating is formed by a calcium phosphate and comprising the steps of:
- a) preparing an aqueous solution comprising:
- 10 i) from 0.3 M to 0.7 M of  $\text{Ca}(\text{OH})_2$ ;
- ii) from 0.1 to 0.5M of  $\text{H}_3\text{PO}_4$ ; and
- iii) from 0.7 M to 1.3 M of lactic acid;
- wherein the solution has a pH of from 3.00 to 4.5 and has a temperature of from 70°C to 90°C;
- 15 b) submerging a porous substrate with a desired shape into the solution of step a) for at least 30 minutes.
2. Process for coating, according to claim 1, **characterized by** the fact that the calcium phosphate coating is selected from the group comprising monetite, brushite, tricalcium phosphate and mixtures thereof.
- 20 3. Process for coating, according to claim 1, **characterized by** the fact that the temperature is 80°C.
4. Process for coating, according to claim 1, characterized by the fact that the time is 60 minutes.
5. Process for coating, according to claim 1, **characterized by** the fact that the aqueous solution further comprises metallic ion, fluoride, chloride and mixture thereof.
- 25 6. Process for coating, according to claim 1, **characterized by** the fact that the porous substrate is a polymeric sponge.
7. Process for coating, according to claim 6, **characterized by** the fact that the polymeric sponge comprises anionic groups on its surface.
- 30

8. Process for coating, according to claims 1 to 7, **characterized by** the fact that it comprises an additional step c.1) of removing the coated substrate  
5 from the solution and drying the substrate.

9. Process for coating, according to claims 1 to 7, **characterized by** the fact that it comprises an additional step c.2) of elevating the pH of the solution up to 13,00 at a temperature range of from 50°C to 90°C for at least 20 hours.

10. Process for coating, according to claim 9, **characterized by** the fact  
10 that the final pH is 12,5.

11. Process for coating, according to claim 9, **characterized by** the fact that the temperature is 60°C.

12. Process for coating, according to claim 9, **characterized by** the fact that it comprises an additional step d.1) of removing the coated substrate from  
15 the solution and drying the substrate.

13. Process for coating, according to claims 8 and 12, **characterized by** the fact that the drying step comprise temperatures higher than 500°C.

14. Porous mineral product **characterized by** the fact that it is produced by the process described in claims 1 to 13.

20 15. Porous mineral product, according to claim 14, **characterized by** the fact that it is used for bone regeneration.

Figures

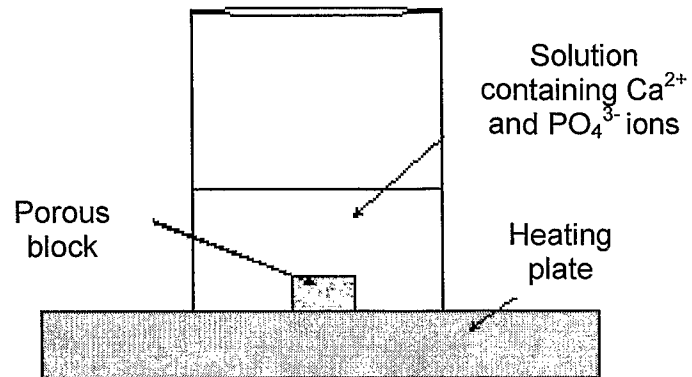


Figure 1

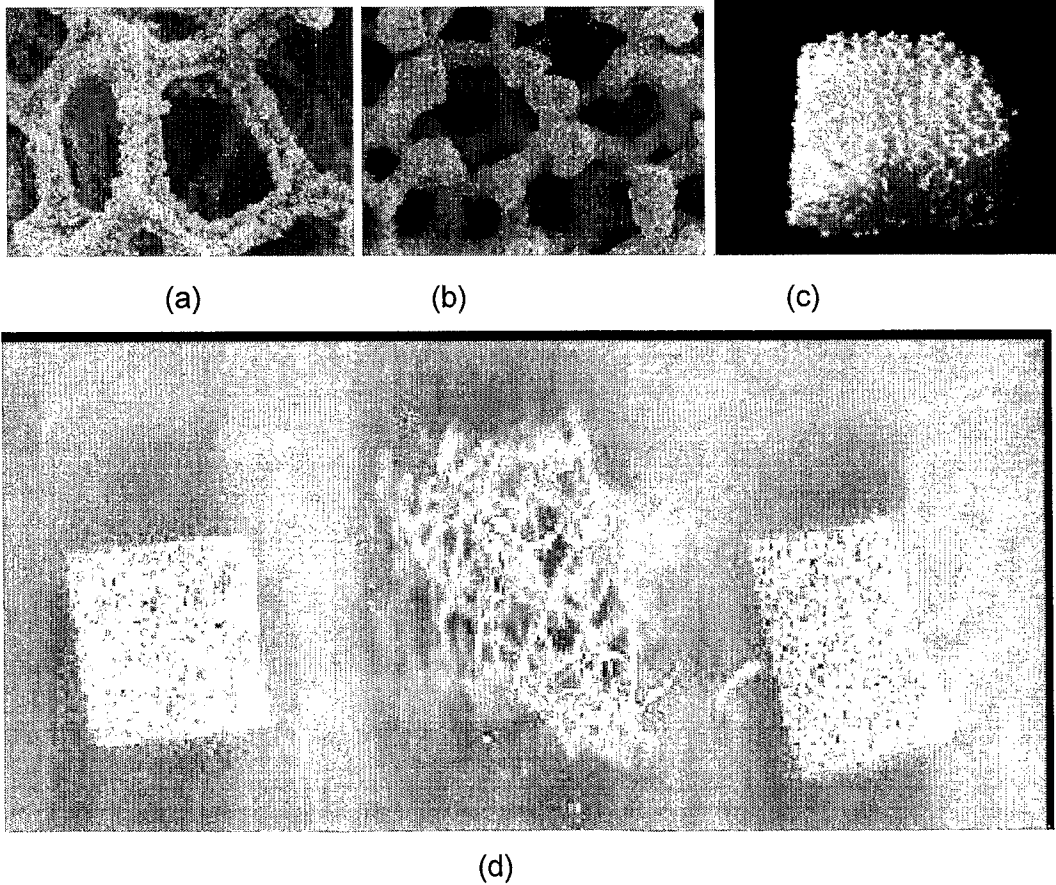


Figure 2

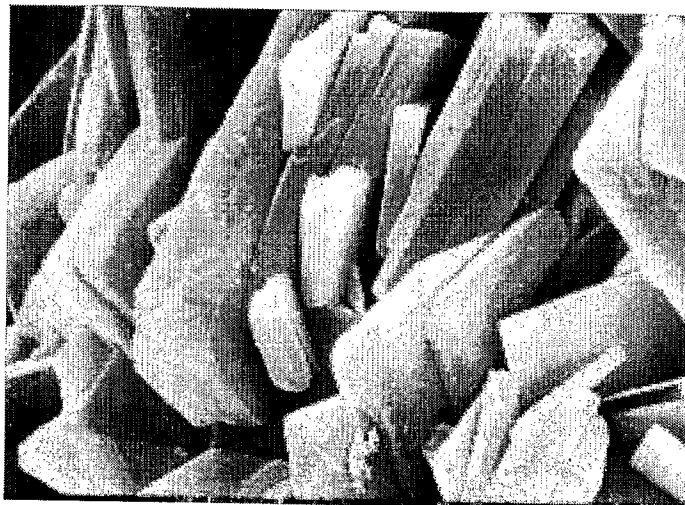


Figure 3

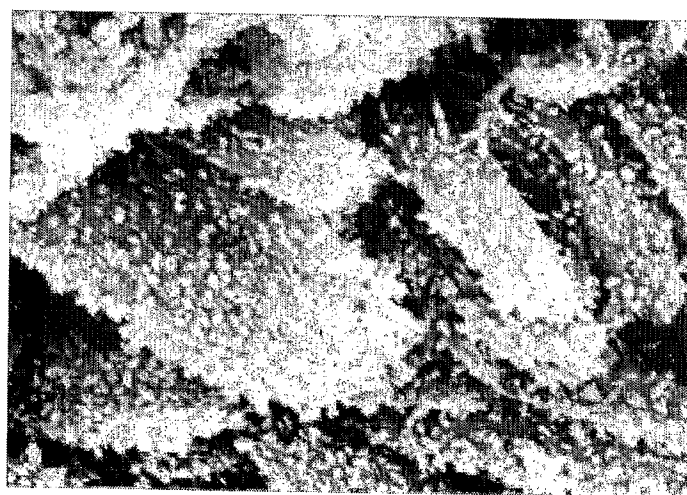


Figure 4

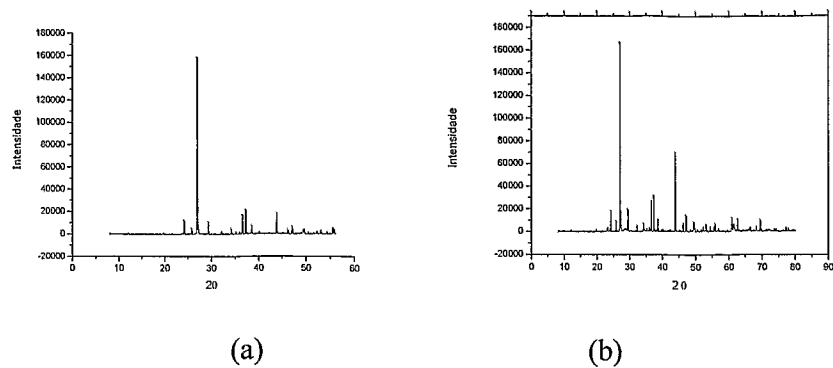


Figure 5

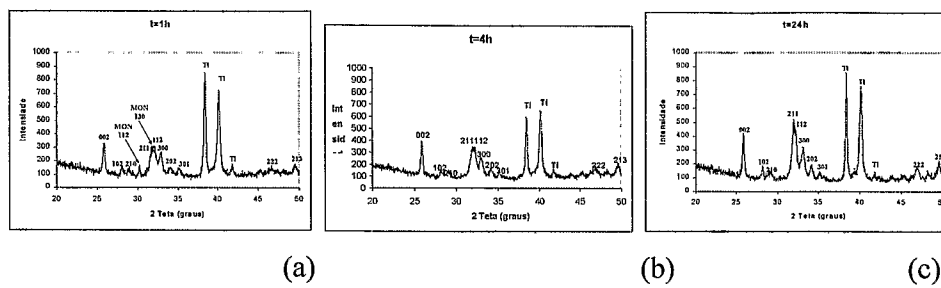


Figure 6

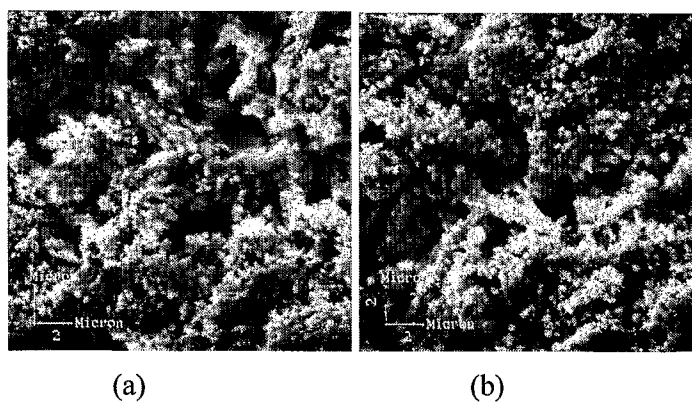


Figure 7



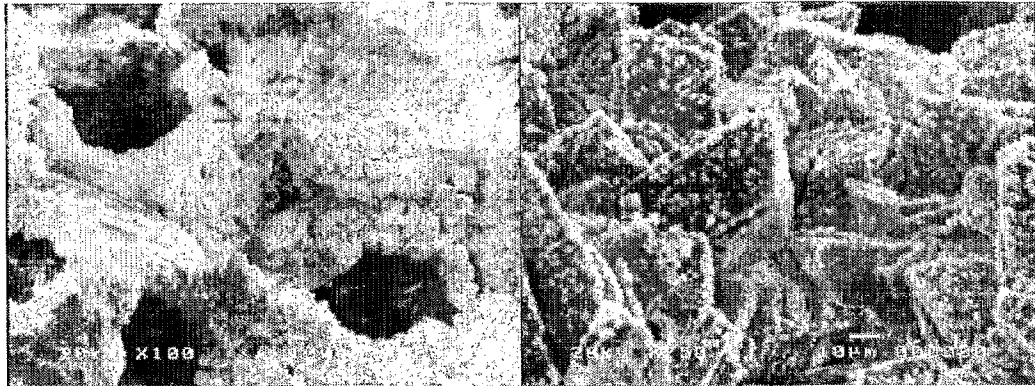


Figure 8

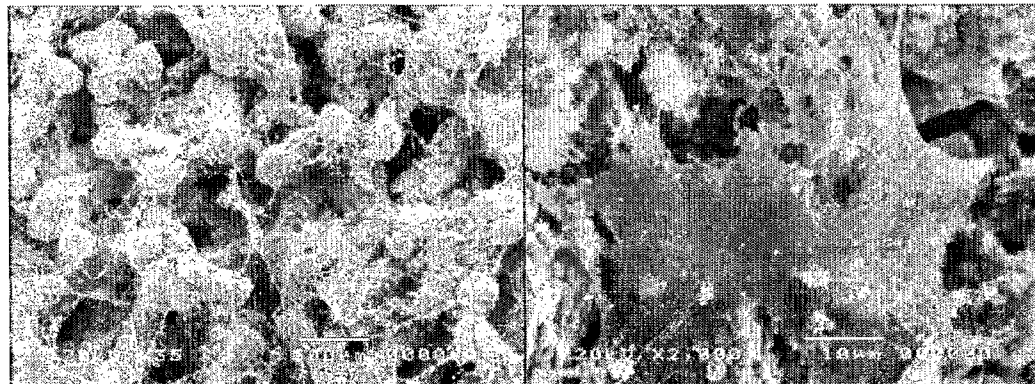


Figure 9

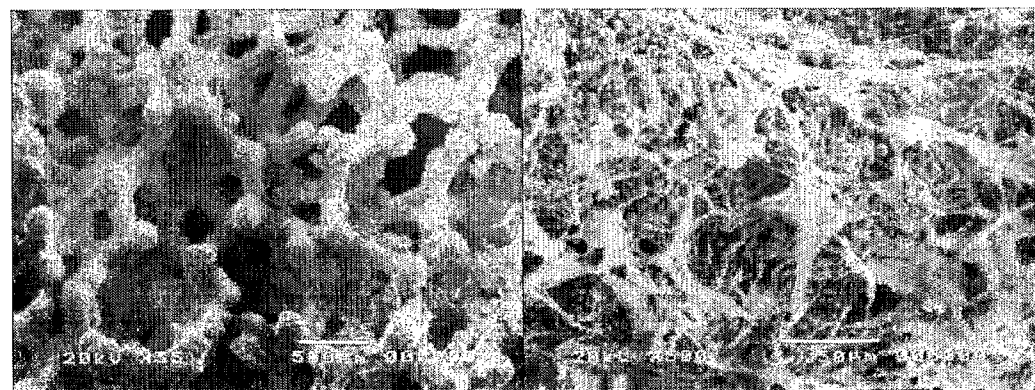


Figure 10

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/BR 2007/000110

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC <sup>8</sup> : <b>A61L 27/32</b> (2006.01); <b>A61F 2/00</b> (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC <sup>8</sup> : A61L, A61F Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI, EPODOC		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5047031 A (CONSTANZ B.), 10 September 1991 (10.09.1991) <i>Column 1, lines 51 - 65, column 3, line 1 - column 5, line 30; column 8, lines 3 - 11; exampla 1; claims 1, 7, 10-14.</i>	1-7,17-19
Y	--- " --- --	7,8
X	US 6991803 B2 (SAPIESZKO R. et al.), 31 January 2006 (31.01.2006) <i>Figures 2, 5-10, column 6, lines 16 - 58; column 8, lines 22 - 67; column 10, line 66 - column 11, line 11; column 23, line 17 - column 25, line 25; examples 1, 13,15, claims 1 - 5.</i>	1-6, 17-19
Y	--- " --- --	7,8
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 20 August 2007 (20.08.2007)		Date of mailing of the international search report 7 September 2007 (07.09.2007)
Name and mailing address of the ISA/ AT <b>Austrian Patent Office</b> Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 / 1 / 534 24 / 535		Authorized officer <b>BAUMSCHABL F.</b> Telephone No. +43 / 1 / 534 24 / 459

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/BR 2007/000110

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5458863 A (KLASSEN R.), 17 October 1995 (17.10.1995) <i>Column 1, line 61 - column 3, line 10; claims.</i>	1 - 8, 17 - 19
Y	--- " --- --	7,8
X	US 2006/0013894 A1 (YAMAMOTO A. et al.), 19 January 2006 (19.01.2006) <i>[0039] - [0041], [0044], [0045], page 6, table 5, claims 1 - 4,7,9,10.</i> ---	1 - 6, 17 - 19.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT2007/BR2007/000110

Patent document cited in search report			Publication date	Patent family member(s)	Publication date
US	A	5047031		US A 5952010	1999-09-14
				US A 6002065	1999-12-14
				US A 6005162	1999-12-21
				US A 5900254	1999-05-04
				US A 5820632	1998-10-13
				US A 5962028	1999-10-05
US	A	6991803		MX A PA05003455	2006-02-10
				US A1 2006039951	2006-02-23
				WO A1 2004030655	2004-04-15
				EP A1 1545466	2005-06-29
				CA A1 2500722	2004-04-15
				AU A1 2003277270	2004-04-23
US	A	5458863		US A 5458863	1995-10-17
US	A	2006013894		JP A 2006025915	2006-02-02
				US A1 2006013894	2006-01-19