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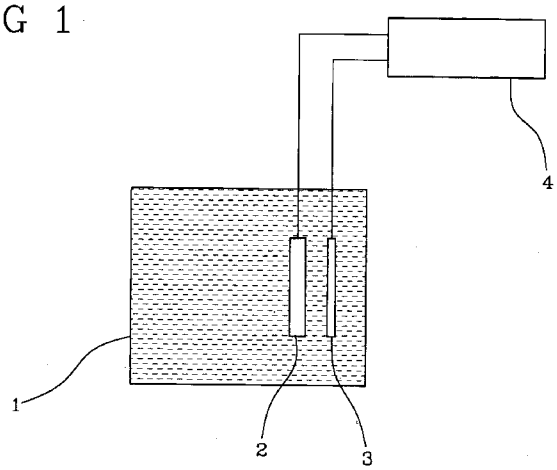
(54) Title: PROCESS FOR COATING A SURFACE OF A METAL ELEMENT TO INCREASE OSTEOINTEGRATION THEREOF AND PROSTHETIC DEVICE INCLUDING SAID ELEMENT

FIG 1



(57) Abstract: A process for coating a surface of a metal element, in particular a metal element of a prosthetic device, comprising: providing an electrolytic bath comprising: at least one collagen, calcium ions and phosphate ions; immersing into said electrolytic bath at least one cathode and at least one anode, said at least one cathode comprising the metal element to be coated; applying electric direct current to said electrolytic bath so as to obtain on said surface an electrolytic co-deposition of collagen with a calcium-phosphate compound comprising hydroxyapatite. The coating layer thus obtained has a high biocompatibility, so as to obtain an interface between metal element and bone structure which enables a faster new bone formation and a bone integration strengthening in time.

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"PROCESS FOR COATING A SURFACE OF A METAL ELEMENT TO INCREASE OSTEOINTEGRATION THEREOF AND PROSTHETIC DEVICE INCLUDING SAID ELEMENT"

DESCRIPTION

The present invention relates to a process for coating at least one metal element of a prosthetic device, in order to obtain a high bone integration between the surface of the metal element and the recipient bone structure, and to the prosthetic device including such coated metal element.

Prosthetic device are for instance: implants, synthetic means, external fastening systems, stabilizing systems, melting cages, dental implants, screws, to be used to clinical/surgical purposes in orthopedics, maxillo-facial surgery and neurosurgery.

The human bone is one of the most complex examples of biomineralized material existing in nature. It is mainly made up of inorganic components such as hydroxyapatite (HA) and water (70-80%), and of organic components such as type I collagen, proteoglycanes and other non-collagenous proteins (20-30%). During bone formation, hydroxyapatite nanocrystals get in close contact with fibrils of type I collagen and generate a nanostructured, regular set with hierarchical structure having given mechanic and elastic properties that

are able to support all organs of the human body. Such deposition mechanism of hydroxyapatite nanocrystals in close contact within collagen fibrils is mediated by competent cells such as fibroblasts and osteoblasts.

The loss of bone substance with consequent need of restoring the lacking volume or the request of increasing the existing bone volume is one of the most relevant problems in orthopedics, maxillo-facial surgery and neurosurgery. Several solutions were proposed to face the lack of bone volume, in particular various substances of natural, inorganic synthetic or merely synthetic origin were used as bone substitute. However, the extraordinary mechanical behavior of natural bone due to its nanocomposite hierarchical structure has been shown by many authors to be hardly achievable with any other type of biomaterial. Therefore, the ideal bone substitute is still autologous bone taken from the patient himself from a donor site. However, this practice is not without risks for the patient, which often result in the resorption of the bone implant itself and frequent appearance of painful symptoms in the site where the bone graft was taken.

Recent studies have shown that the use of allogenous bone from a bank of tissues of human origin, processed and made inert by chemical-physical processes, can be

an alternative to the use of autologous bone. However, also this practice can give rise to risks related to the development of infective illnesses or immune reactions.

New biomaterials acting as bone substitutes have been studied and proposed for clinical use, some of them have shown positive results after clinical examinations on humans. Such materials do not only have high biocompatibility properties (inert biomaterials), but also have biomimetic characteristics, i.e. chemical and chemical-physical properties that are similar to humane bone and can activate biologic mechanisms (bioactivity) with recipient bone tissues and cell components contained therein, thus favoring new bone formation and bone strengthening processes. Once their stimulating action for new bone formation is completed, these materials are sometimes completely resorbed leaving only the newly formed bone.

The kinetics of bone formation and bone strengthening require quite long times, and immediately after surgery and after a certain period, in particular for the reconstruction of some bone structures such as long bones, the immediate load cannot be applied without a mechanical support. That is why complementary stabilizing and mechanically fastening means of metal na-

ture ensuring an immediate load are often used. The use of complementary metal structures in bone reconstruction procedures is not limited only to reconstructions without an immediate mechanical load, but are very often an integral part of bone regeneration such as for instance in dental/maxillo-facial surgery, wherein the positioning of titanium screws becomes in time a single body with the recipient bone, giving rise to an anatomical system that can house artificial dental elements for recovering the loss of dental elements.

The use of metal devices based on chrome-cobalt alloys or more frequently on titanium alloys has enabled to obtain good results in terms of osteointegration or bone integration, wherein osteointegration (a concept introduced by Branemark at the end of the 60s and later developed in the 80s with the oncoming of new histological techniques) refers to the direct, structural and functional union between the recipient vital bone and the surface of a prosthetic device subjected to load. The implementation and preservation of bone integration depend on bone healing, repairing and remodeling capacity.

It is clear that a complete osteointegration of metal with the recipient bone is extremely important, since

anatomical and morpho-functional results of many bone reconstruction surgical operations depend on it. For instance, if a complete bone integration with the healthy bone is not obtained in dental surgery, there is the risk of mobilization of prosthetic implants.

Titanium alloys (mainly Ti_6Al_4V alloys and Ti_6Al_7Nb alloys) are preferred with respect to other metal alloys not only thanks to their easy processing, but especially thanks to their high biocompatibility, high mechanical resistance, resistance to corrosion, low tendency to induce bone resorption and to their elastic properties which, if compared with other metal alloys, are more similar to those of human bone (relatively low elastic modulus of 100 GPa with respect to over 200 GPa of alternative materials, stainless steels and cobalt alloys).

There are several decisive factors for the achievement of a good osteointegration of implantable devices for orthopedic surgery, neurosurgery and maxillo-facial surgery: beyond biomechanical factors related to the geometry and shape of the implant, the topographical and chemical nature of the implant surface interfacing with healthy bone tissue and its components making up the connective tissue, first of all cell component, are important. The mechanism of bone formation on the

implant surface begins with a cell chemotaxis towards the interface bone-implant and migrations of other factors (macromolecules, chemical mediators, proteins, nutrients etc.) with the formation first of a coagulum and then of granulation tissue that can prepare an ideal bed for the transformation of undifferentiated parent cells (mesenchymal cells present in bone marrow) into mature cells that can synthesize the mineral bone matrix in the final neoossification stage. Eventually, the result of a good bone integration is inevitably determined by a perfect quality of the recipient bone. As a matter of fact, metal implants housed in patients suffering from both senile and secondary osteoporosis or from other degenerative illnesses of the skeletal structure very often fail.

There are today particular techniques and treatments which can implement bone integration properties, therefore durable stability, of metal implants by modifying surface characteristics (e.g. modification of rugosity) thanks to chemical or physical process enabling the deposition of thin ceramic films onto the contact surfaces with the bone. These treatments, beyond achieving a higher bone integration and a long stability, can also give a higher biocompatibility to the prosthesis and limit the phenomenon of release of

metal ions due to mechanical stress and frictions. A particular care has been reserved to the surface treatment of knee and hip prostheses, wherein fastening systems should have particular macrorugosity and geometries, whereas in order to reduce stress shielding prosthesis surfaces should be treated with special coatings including fills of titanium nitride (TiN) or ceramized TiNbNi. These particular surface treatments can be obtained in the first case by sandblasting or mechanical processing, or by deposition of microparticles of ceramic or metallic material thanks to specific coating processes (Ti-plasma spray, deposition of sintered HA microspheres, metal fiber meshes). In the case of outer coatings of metal surfaces interfacing with other joint surfaces, these coatings are obtained by chemical-physical processes such as CVD (Chemical Vapor Deposition) and PVD (Physical Vapor Deposition), which allow to obtain ultrathin (size of some tens of nm) and resistant coatings that are able to damp mechanical stresses due to friction between adjacent joint surfaces, thus preventing the release of ions that may induce inflammatory reactions on adjacent tissues.

If on one side these techniques can ensure a faster primary bone integration (e.g. in maxillo-facial sur-

gery 3-6 months), in the long run they can be intrinsically subject to risks of debridement that may alter the mechanical and surface properties of the metal device compromising the biocompatibility, stability and bone integration thereof (inflammation on the interface prosthesis-connective tissue with formation of surface lacunae and osteolysis).

Other complementary systems, therapies and techniques can be used so as to promote bone integration (laser therapy, ultrasounds etc) by means of a bone stimulation that can make parent cells proliferate and differentiate into competent cells for the formation of bone matrix.

It is known that a direct nucleation in an aqueous solution of hydroxyapatite (HA) nanocrystals inside collagen fibers enables to obtain a biomimetic composite with characteristics similar to human bone. HA nucleation inside collagen fibers induces the carbonatation of HA nanocrystals in B position with replacement of PO_4^{3-} ion with CO_3^{2-} ion, similarly to what occurs in the process of deposition of human apatite, preventing the deposition in A position wherein CO_3^{2-} ion would replace OH^- group as occurs in synthetic carbonate-apatite. The close analogy in terms of microstructure and chemical composition of the nanocomposite colla-

gen-apatite with human bone can result in a bioactive structure which, once deposited onto a metal surface, is able to modulate the kinetics of new bone formation and bone remodeling. Nanostructured composites of calcium phosphates/collagen are apt to be deposited onto metal surfaces. However, traditional coating methods such as plasma spray, CVD (Chemical Vapor Deposition) and PVD (Physical Vapor Deposition) cannot be applied to this type of material, especially due to the fibrous shape of collagen.

As is described in the related literature, many difficulties have been found when making coatings of composite of calcium phosphate/collagen uniform and homogeneous on metal surfaces by electrolytic deposition. For instance, the article by H. Schliephake et al., "Biological performance of biomimetic calcium phosphate coating of titanium implants in the dog mandible" published in *J Biomed. Mater. Res.*, (2003) 64, pages 225-234, discloses a study on the in vivo effects of titanium implants coated with various materials. In particular, use was made of prosthetic titanium implants coated with a bioactive multilayer of calcium phosphate/collagen, obtained by electrocrystallization of a solution of calcium phosphate on a titanium electrode onto whose surface collagen had

been previously deposited by partial integration of the latter into a layer of oxides obtained by anodic electrodeposition. Collagen integration has been obtained under galvanostatic conditions using an electrolyte containing collagen under almost physiological conditions (pH 7.4 at 37°C), and subsequent immersion into a solution of collagen for 10 min, so as to obtain a collagen layer with a thickness of about 40 nm. As disclosed by the authors, the total thickness of the multilayer (about 500 nm) and the mineralization degree of collagen were very low.

The article of Y. Fan et al. "A composite coating by electrolysis-induced collagen self-assembly and calcium phosphate mineralization", published in *Biomaterials*, (2004) 26, pages 1623-1632, describes further attempts of electrochemical deposition of multilayer coatings of collagen and calcium phosphate. In particular, it describes the electrolytic deposition onto a silicon substrate of a first layer with a thickness of about 100 μm of calcium phosphate and of a second layer in the form of a gel with a thickness of about 100 μm made from a composite of collagen fibrils with octacalcium phosphate (OCP). The electrolytic deposition was carried out in a solution of $Ca(NO_3)_2$ and $NH_4H_2PO_4$ added with a solution of 1 M NaOH so as to ob-

tain a pH of 4.8-5.3, which was later added with type I collagen in acid solution, followed by a post-adjustment of pH to the values referred to above. The electrolytic deposition was carried out at a constant potential at the cathode of -1, -2 or -3V with respect to the reference cathode (saturated calomelane electrode, SCE).

The Applicant aims at increasing the bone integration capacity of prosthetic devices including at least one metal element by way of a coating process of the element surface with at least one layer of a highly biocompatible material, so as to obtain an interface with the bone structure that enables a faster new bone formation and a bone integration strengthening in time.

The Applicant has found that this problem can be solved by means of a process according to the following claims, wherein a composite of collagen with a calcium phosphate is deposited electrochemically onto the surface of the metal element designed to come in contact with the bone structure, under such process conditions that collagen fibers self-assemble with the inorganic calcium phosphate component near the metal surface, precipitating onto the latter in one mineral phase based on HA, without the addition of alkaline compounds into the electrolytic bath.

According to a first aspect, the present invention therefore relates to a process for coating a surface of a metal element, comprising:

providing an electrolytic bath comprising at least one collagen, calcium ions and phosphate ions;

immersing into said electrolytic bath at least one cathode and at least one anode, said at least one cathode comprising the metal element to be coated;

applying electric direct current to said electrolytic bath so as to obtain on said surface an electrolytic co-deposition of collagen with a calcium-phosphate compound comprising hydroxyapatite.

According to another aspect, the present invention relates to a prosthetic device comprising at least one metal element, said metal element having at least one surface coated with at least one layer that can be obtained by electrolytic co-deposition of a collagen with a calcium phosphate compound comprising hydroxyapatite.

According to a preferred embodiment, at the beginning of co-deposition the electrolytic bath has a pH of from 3.0 to 4.5, preferably from 3.5 to 4.0, said pH rising in proximity to the cathode to a value above 8, generally from 10 to 12, during the co-deposition process without addition of any alkaline compound. At

the end of the electrolytic co-deposition process, the electrolytic bath generally has a pH of from 4.0 to 6.0, preferably from 4.5 to 5.5.

According to a preferred embodiment, the electrolytic co-deposition is carried out with a substantially constant direct current. Preferably, the direct current is kept at relatively low values so as to avoid the removal of deposited material due to an excessive development of gaseous hydrogen at the cathode. Generally, the direct current has a value of from 10 to 60 mA, more preferably from 30 to 40 mA.

Preferably, collagen is type I collagen. Preferably, collagen undergoes a preliminary step of enzymatic digestion in order to remove telopeptides present on collagen molecules, which might prevent the self-assembly of the protein on the metal surface.

Preferably, collagen is present in the electrolytic bath as a suspension and has in general an initial concentration of from 0.005 to 0.05% weight/volume, preferably from 0.01 to 0.02% weight/volume.

Preferably, calcium ions are added to the electrolytic bath as a soluble salt under reaction conditions, e.g. calcium nitrate. The initial concentration of phosphate ions in the electrolytic bath is generally of 0.01 to 0.1 moles/liter, preferably 0.02 to 0.06

moles/liter.

Preferably, phosphate ions are added to the electrolytic bath as a soluble salt under reaction conditions, e.g. ammonium dihydrogenophosphate.

The initial concentration of phosphate ions in the electrolytic bath is generally of from 0.01 to 0.10 moles/liter, preferably from 0.02 to 0.04 moles/liter. In the framework of the present invention, the term "phosphate ions" includes both phosphate PO_4^{3-} ions and hydrogenophosphate HPO_4^{2-} as well as dihydrogenophosphate $H_2PO_4^-$ ions that might be present in equilibrium with PO_4^{3-} ions in variable amounts depending on pH.

Preferably, the metal element comprises titanium or an alloy thereof, e.g. Ti_6Al_4V alloys or Ti_6Al_7Nb alloys.

By implementing the process according to the present invention, the thickness of the collagen and hydroxyapatite layer can be varied within wide intervals, from a thickness of few nm, e.g. from 10 nm to 500 nm, up to higher thickness values, generally from 50 μm to 500 μm, preferably from 100 μm to 200 μm. The thickness of the collagen and hydroxyapatite layer is preferably not higher than 500 μm, since higher thickness values may cause delamination phenomena that would result in surface irregularities jeopardizing the bone integration capacity of the treated surface.

Preferably, the calcium phosphate compound comprises at least 90% by weight, more preferably at least 95% by weight, of hydroxyapatite. Preferably, hydroxyapatite is present as lanceolate, flat crystals (biomimetic morphology) with an average size of the longest side below 300 nm, more preferably from 100 to 200 nm.

The layer obtained by electrolytic co-deposition according to the present invention generally comprises: from 10 to 90% by weight of hydroxyapatite and from 10 to 90% by weight of collagen. More preferably, said layer comprises: from 60 to 70% by weight of hydroxyapatite and from 30 to 40% by weight of collagen.

The present invention will now be disclosed thanks to some examples of embodiment, which are provided as mere non-limiting examples of the scope of the invention.

The figures accompanying the present description show:

Fig. 1: a schematic representation of a device for implementing the electrolytic co-deposition according to the present invention;

Fig. 2: SEM image of the coating obtained by co-deposition of collagen and hydroxyapatite according to the invention after 5 min. of electrodeposition;

Fig. 3: TEM images of the coating obtained by co-deposition of collagen and hydroxyapatite according to

the invention; (a) and (b) after 5 min. of electrodeposition, (c) and (d) after 30 min. of electrodeposition;

Fig. 4: FTIR spectrum of the coating obtained by co-deposition of collagen and hydroxyapatite according to the invention after 30 min. of electrodeposition (spectrum A), compared with the FTIR spectrum of a bone tissue (spectrum B);

Fig. 5: X-ray diffractogram obtained by co-deposition of collagen and hydroxyapatite according to the invention after 30 min. of electrodeposition; the supplement contains a portion of a X-ray diffractogram of a bone tissue by way of comparison;

Fig. 6: SEM images of self-assembled collagen fibers according to the invention, after decalcification with EDTA/glutaraldehyde.

EXAMPLE 1.

Materials used.

All reagents were chemical grade.

Type I collagen used in the experiments was extracted from horse tendon using a standard manufacturing method wherein animal tissues and the derived raw tissue were subjected to strict medical-veterinary controls. After completely removing the synovial membrane, the tissue was finely cut up and suspended in

an aqueous HCl solution at pH 2.5, then digested with pepsin for 24 h. After enzymatic digestion collagen was precipitated raising pH to 5.5 by addition of a NaOH solution and then subjected to washing cycles with distilled water, then treated with 1 M NaOH for 1 h so as to remove any glycoside residue and ensure a complete viral deactivation. At the end of the procedure, it was treated in an ambient at pH 5.5 by addition of HCl. Short before the deposition process, collagen fibers were homogeneously resuspended (1% w/w) in 0.3% acetic acid (w/w).

99.7% titanium laminas were obtained from Sigma (code 267503-25.2G) and then cut into strips sized 15 mm x 25 mm.

Process conditions

The electrolytic deposition processes described below were carried out with a two-electrode electrochemical system schematically shown in Figure 1, comprising an electrolytic bath (1) in which calcium ions, phosphate ions and collagen as suspension in the aqueous medium are present; a cathode (2) and an anode (3) are immersed in the electrolytic bath (1), between the latter an electric direct current is applied, which is controlled by means of a potentiostat (4) used as galvanostat (Amel model 552-Potentiostat/Amel model 721-

Integrator). The cathode was made up of a titanium foil sized 15 mm x 25 mm. Before electrolytic deposition, the titanium foil was cleaned by ultrasonic treatment in acetone bath and then in distilled water till every residue was removed.

The electrolytic bath was prepared by dissolving in distilled water $Ca(NO_3)_2$ and $NH_4H_2PO_4$ until a concentration of 42 mM of $[Ca^{2+}]$ and 25 mM of PO_4^{3-} was achieved. A suspension of type I collagen prepared as described above was added to the electrolytic bath in a concentration of 0.012% w/v. The initial pH of the electrolytic bath was of about 4.0. At the end of co-deposition the pH of the electrolytic bath was of about 5.0-5.5.

During the electrolytic deposition, the electrochemical cell containing the electrolytic bath was kept at room temperature while applying a basically constant current of 34 mA. The duration of the deposition process of the composite coating of collagen/HA was fixed at 5 seconds, 30 seconds, 5 minutes, 30 minutes.

Taking into account the electrolytes present in the solution, the main reactions occurring at the titanium cathode when the current passes through the electrodes are the following:

1) $O_2 + 2\ H_2O + 4\ e^- \rightarrow 4\ OH^-$

2) $2\ H_2PO_4^- + 2\ e^- \rightarrow 2\ HPO_4^{2-} + H_2$

3) $HPO_4^{2-} + 2\ e^- \rightarrow 2\ PO_4^{3-} + H_2$

4) $2\ H_2O + 2\ e^- \rightarrow H_2 + 2\ OH^-$

5) $2H^+ + 2\ e^- \rightarrow H_2$

It is believed that these reactions can increase the pH value in the cathode area so as to create suitable conditions for the co-deposition of collagen with hydroxyapatite without adding any alkaline compound. Therefore, we can assume that the collagen begins to self-assemble only near the cathode, where a local rise of pH is obtained by effect of the electrochemical process, and not in the whole mass of the bath as a result of the addition of OH^- ions.

The initial pH of the electrolytic bath is adjusted to a value below the isoelectric point of collagen, so as to enable the migration of the positively charged molecule to the cathode. The reactions occurring at the cathode as described above, with the production of OH^- ions, enable to go beyond the isoelectric point of collagen and at the same time to generate suitable conditions for the deposition of calcium phosphate, with the resulting simultaneous self-assembly of collagen and association thereof with the mineral phase, so as to deposit onto the cathode surface the desired composite.

Characterization of the coatings.

Various samples obtained according to Example 1 were characterized as follows.

Infrared microscopy spectra (FTIR) were obtained with a Perkin-Elmer infrared spectrophotometer mod. Spectrum One FT-IR with an associated Perkin-Elmer microscope (Perkin-Elmer Autoimage microscope). Spectral resolution was of 4 cm^{-1}, spatial resolution was of 100x100 µm, whereas the spectrum was the result of 32 scans. The basic line of the spectrum was obtained from a region without sample. The specific areas to be analyzed were identified by vision with a camera located on the microscope.

Scanning electron microscope (SEM) images were obtained with a Philips 515 microscope. The titanium foil subjected to electrochemical coating was suitably placed and treated with a coating of colloidal gold in a time range of from 30 to 180 seconds at a voltage of 30 mV.

The analysis with transmission electron microscope (TEM) was carried out with a Philips 420T microscope. A sample of pulverized coating was suspended in bi-distilled water and a drop of the suspension thus obtained was deposited onto a perforated carbon foil supported by a copper microgrid.

X-ray diffractometry was employed for determining the crystallinity degree by using an Analytical X'Pert Pro diffractometer and a α CuK radiation generated at 40 kV and 40 mA. The instrument was configured at 1° of divergence and 0.2 mm of receiving slit. The crystallinity degree was evaluated by applying the following formula:

Crystallinity [%] = 100 X Σ $I_{net.}$/(Σ $I_{tot.}$ - $Bgr._{const.}$).

The size of the single crystal was determined by applying Scherrer's formula:

$$L_{(0,0,2)} = \frac{0.94\lambda}{\left[\cos\theta(\sqrt{\Delta r^2 - \Delta_0^2})\right]}$$

wherein θ represent the angle of diffraction, Δr and Δ_0 represent the amplitude in radians of the angle of reflection at mid height for the reference peak of the final product and of hydroxyapatite, respectively, at λ=1.5405 Å.

Comments on the results obtained.

SEM observation of the coating obtained after an electrodeposition with a duration of 5 minutes (see Fig. 2) shows the presence of a mineral phase closely associated with collagen fibrils. The important role of strengthening of the inorganic component by the collagenous fibrous network can be inferred from the image. By increasing electrodeposition time to 30 minutes,

the SEM image shows collagen fibers as completely immersed in the inorganic nanocrystalline phase.

The analysis of the FTIR spectrum of the same coating (see Fig. 4) shows peaks of maximum absorption of collagen and of hydroxyapatite. Note the presence in spectrum A of a peak at 1422 cm^{-1} that is wholly similar to the one present in bone tissue (spectrum B), as is typically present in hydroxyapatite of mineralized tissues as a consequence of a B type carbonatation.

The deposition of collagenous fibrils with a different mineralization degree, as shown by TEM images (see Fig. 3), points out that there are hydroxyapatite nanocrystals associated to the collagenous fibril structure whose size changes as a function of deposition time. The nanocrystals in the initial deposition stage (deposition time 5 sec., images (a) and (b)) are sized 40±10 nm and have an irregular shape. After a deposition time of 30 minutes, the hydroxyapatite crystals take a needle-like shape with a length of 160±20 nm and a width 60±10 nm (images (c) e (d)). During the first stage of coating deposition, crystals seem to be located inside the collagenous fibrils in the less mineralized area of the sample, whereas in the more mineralized area there occurs the opposite phenomenon, i.e. mineralization takes place outside

collagenous fibrils. This phenomenon lets us assume that the self-assembly stage of collagen I molecules deprived of telopeptides, so as to later precipitate in fibril form, occurs simultaneously to the nanocrystallization stage of carbonate-hydroxyapatite. During this process the nucleation of hydroxyapatite nanocrystals and the deposition thereof into collagen fibrils (calcification process) takes place only during the first stage, whereas the nucleation of apatite on fibril surface occurs at a later stage. These two different mineralization processes of collagen fibers basically reproduce the process observed during the preparation of collagen fibers/hydroxyapatite crystals self-assembled in aqueous solution. However, by using the electrodeposition process it is possible not only to control the process, but also to modulate and localize the deposition on the substrate surface.

The X-ray diffractometric analysis (see Fig. 5) of the coating obtained according to the present invention after 30 min. of electrodeposition shows the characteristic peaks of the HA mineral phase at values 2 Theta = 32 and 26. In Fig. 5 the insert shows a portion of the diffractogram of a bone tissue, so as to point out a development that is wholly similar to the one of the sample obtained according to the invention.

In order to check the morphology of collageous fibrils deposited at the titanium electrode after 30 minutes, some samples were decalcified in 10% EDTA solution for 24 hours. This process enables to completely remove the mineral phase surrounding the fibers and improves the characterization and quantification thereof. SEM images concerning the coating on the titanium foil subjected to decalcification are shown in Figure 6. Collagen fibers are shown as deposited homogeneously on the titanium surface without any preferential orientation; this may lead to believe that the mineralization process does not significantly modify the typical morphology of this molecule.

CLAIMS

1. A process for coating a surface of a metal element, comprising:

preparing an electrolytic bath comprising: at least one collagen, calcium ions and phosphate ions;

immersing into said electrolytic bath at least one cathode and at least one anode, said at least one cathode comprising the metal element to be coated;

applying electric direct current to said electrolytic bath so as to obtain on said surface an electrolytic co-deposition of collagen with a calcium-phosphate compound comprising hydroxyapatite.

2. The process according to claim 1, wherein at the beginning of co-deposition the electrolytic bath has a pH of from 3.0 to 4.5, preferably from 3.5 to 4.0, said pH rising in proximity to the cathode to a value above 8, preferably from 10 to 12 during the co-deposition process without addition of any alkaline compound.

3. The process according to claim 2, wherein at the end of the co-deposition process the electrolytic bath has a pH of from 4.0 to 6.0, preferably from 4.5 to 5.5.

4. The process according to any of the preceding claims, wherein the electrolytic co-deposition is car-

ried out with a substantially constant direct current.

5. The process according to claim 4, wherein the direct current has a value of from 10 to 60 mA, preferably from 30 to 40 mA.

6. The process according to any of the preceding claims, wherein said at least one collagen is a type I collagen.

7. The process according to any of the preceding claims, wherein said at least one collagen is subjected to a preliminary stage of enzymatic digestion.

8. The process according to any of the preceding claims, wherein said at least one collagen is present in the electrolytic bath with an initial concentration of from 0.0005 to 0.05% weight/volume, preferably from 0.01 to 0.02% weight/volume.

9. The process according to any of the preceding claims, wherein calcium ions are present in the electrolytic bath in an initial concentration of from 0.01 to 0.1 moles/liter, preferably from 0.02 to 0.06 moles/liter.

10. The process according to any of the preceding claims, wherein phosphate ions are present in the electrolytic bath in an initial concentration of from 0.01 to 0.10 moles/liter, preferably from 0.02 to 0.04 moles/liter.

11. The process according to any of the preceding claims, wherein the metal element comprises titanium or an alloy thereof.

12. The process according to any of the preceding claims, wherein the co-deposition of collagen and the calcium phosphate compound allows to obtain a layer with a thickness of 10 nm to 500 nm.

13. The process according to any of the claims 1 to 11, wherein the co-deposition of collagen and the calcium phosphate compound allows to obtain a layer with a thickness of 50 μm to 500 μm, preferably 100 μm to 200 μm.

14. The process according to any of the preceding claims, wherein the calcium phosphate compound comprises at least 90% by weight, preferably at least 95% by weight, of hydroxyapatite.

15. The process according to any of the preceding claims, wherein hydroxyapatite is present as lanceolate flat crystals with an average size of the longest side below 300 nm, preferably from 100 to 200 nm.

16. The process according to any of the preceding claims, wherein the layer obtained by electrolytic co-deposition comprises: from 10 to 90% by weight, preferably from 60 to 70% by weight, of hydroxyapatite, and from 10 to 90% by weight, preferably from 30 to

40% by weight, of collagen.

17. A prosthetic device comprising at least one metal element, said metal element having at least one surface coated with at least one layer that can be obtained by electrolytic co-deposition of a collagen with a calcium phosphate compound comprising hydroxyapatite.

18. The device according to claim 17, wherein the electrolytic co-deposition is defined according to any of the claims from 1 to 16.

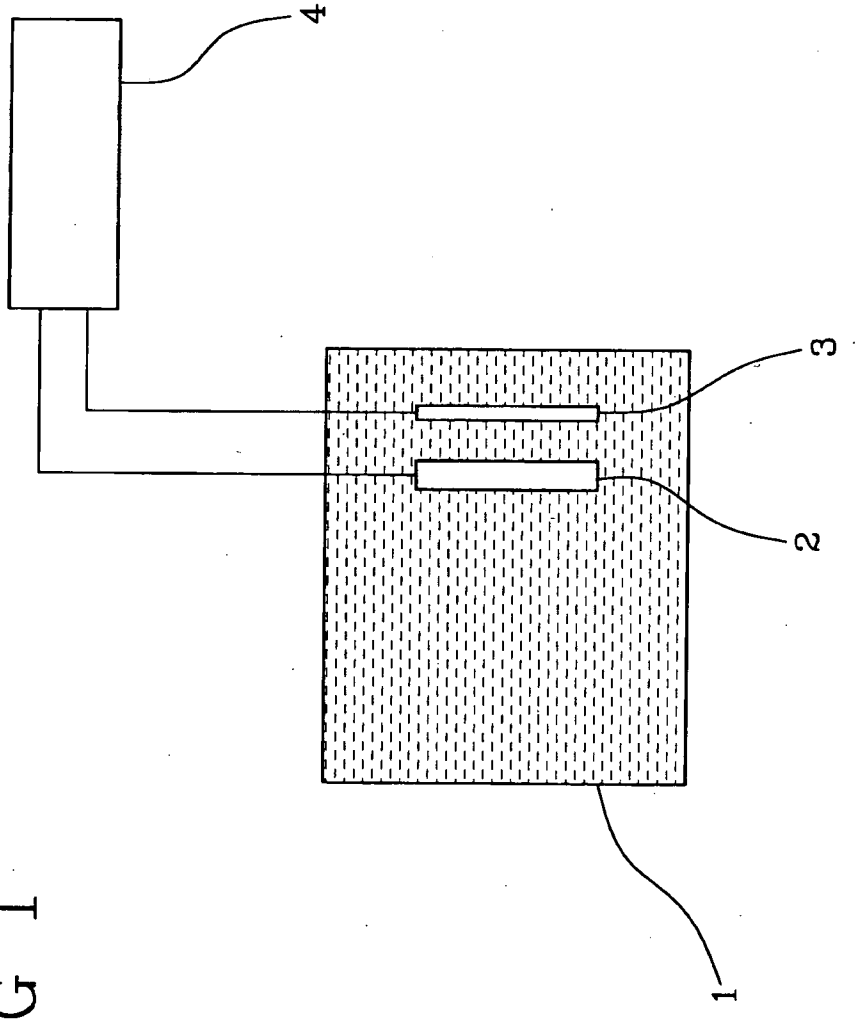


FIG 1

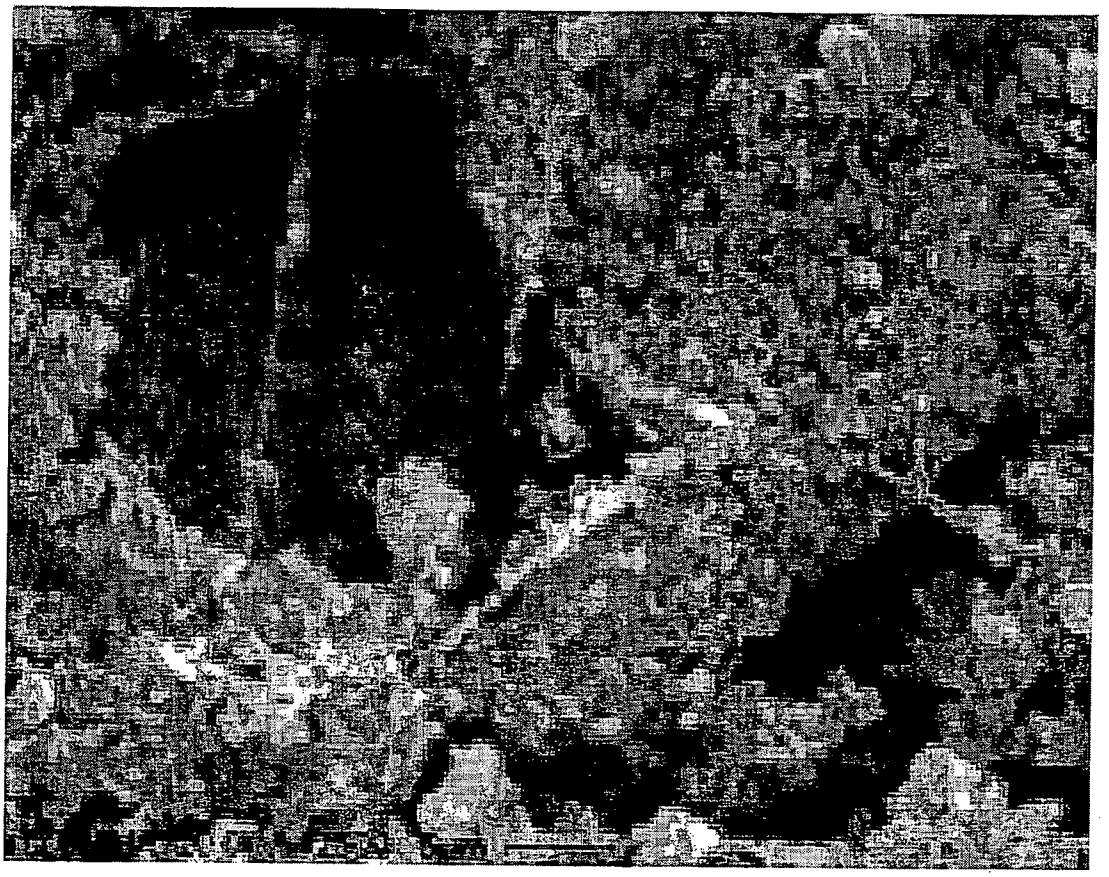


FIG. 2

FIG. 3

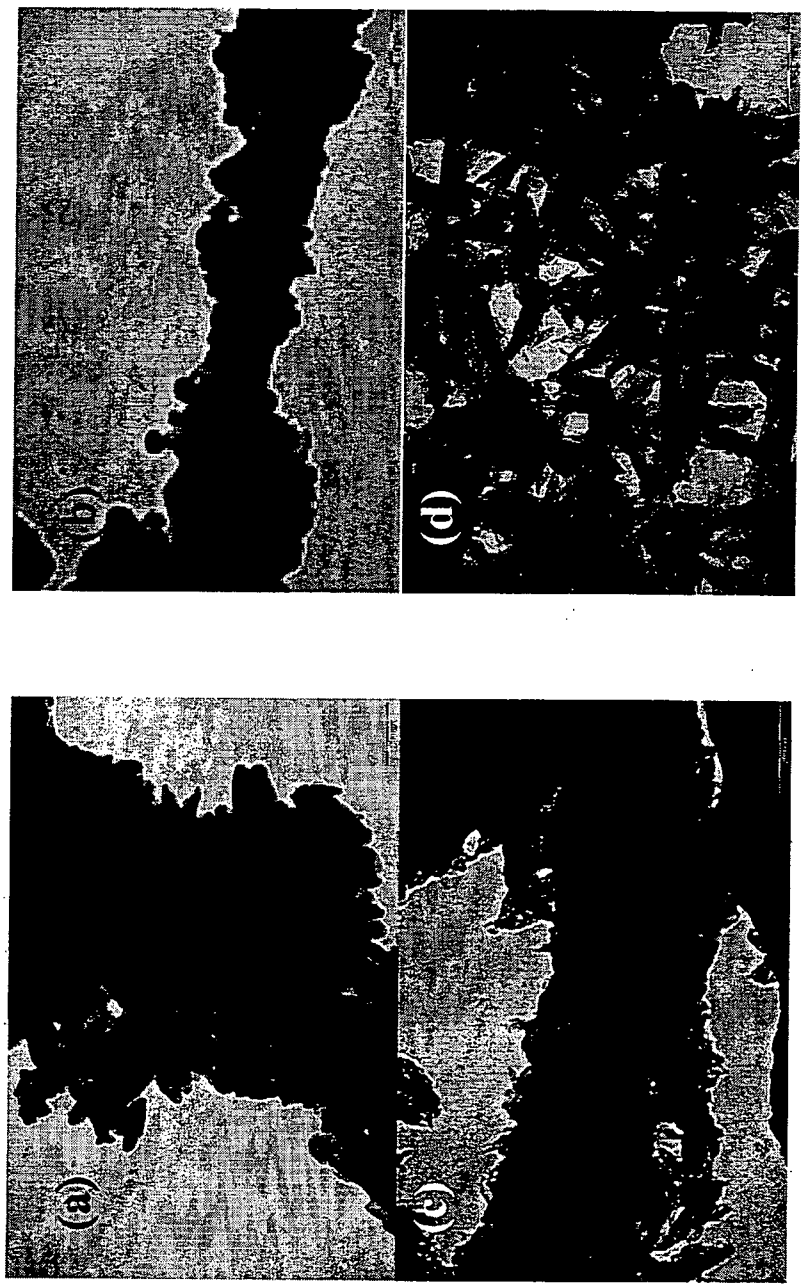


FIG 4

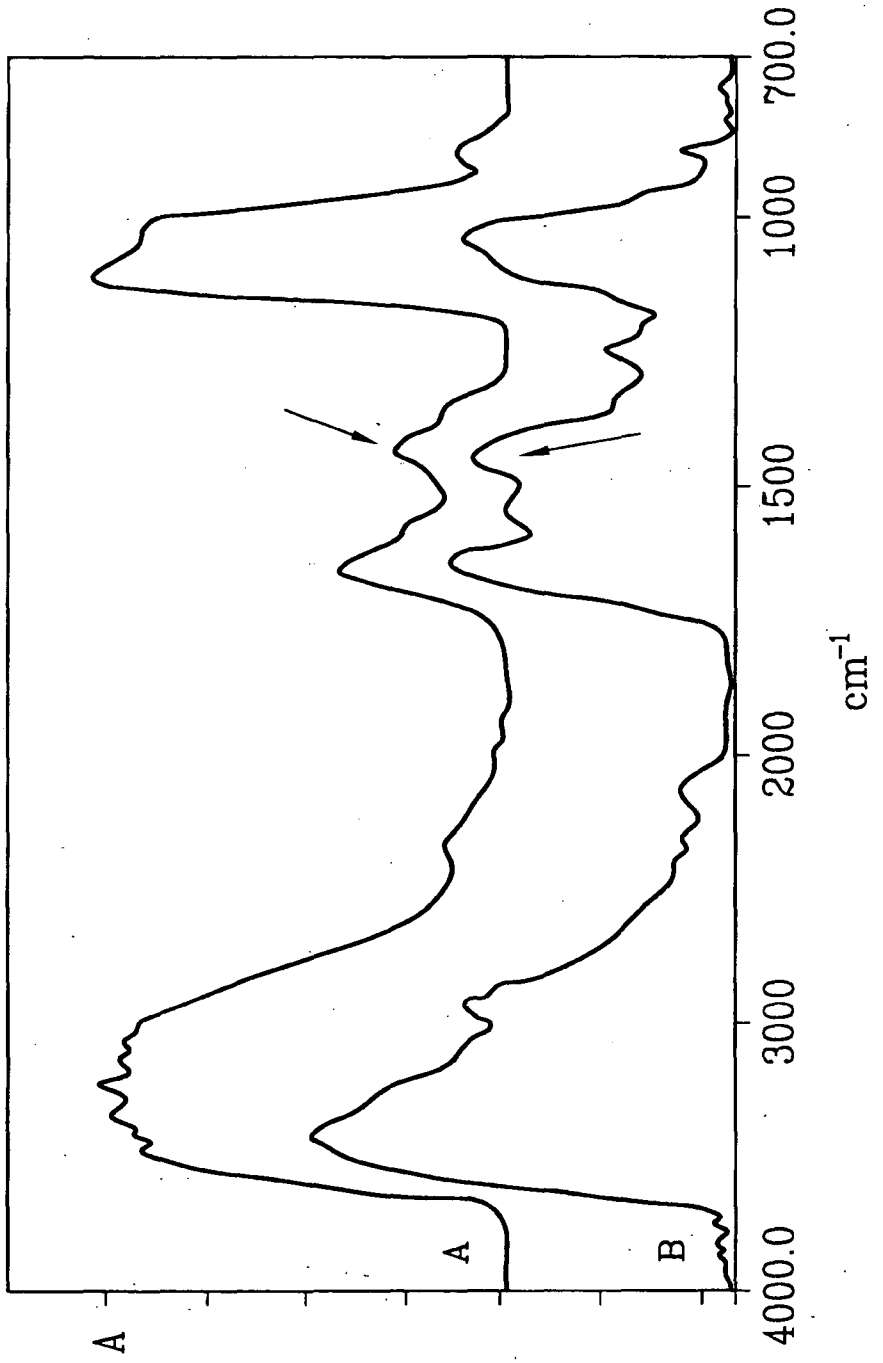
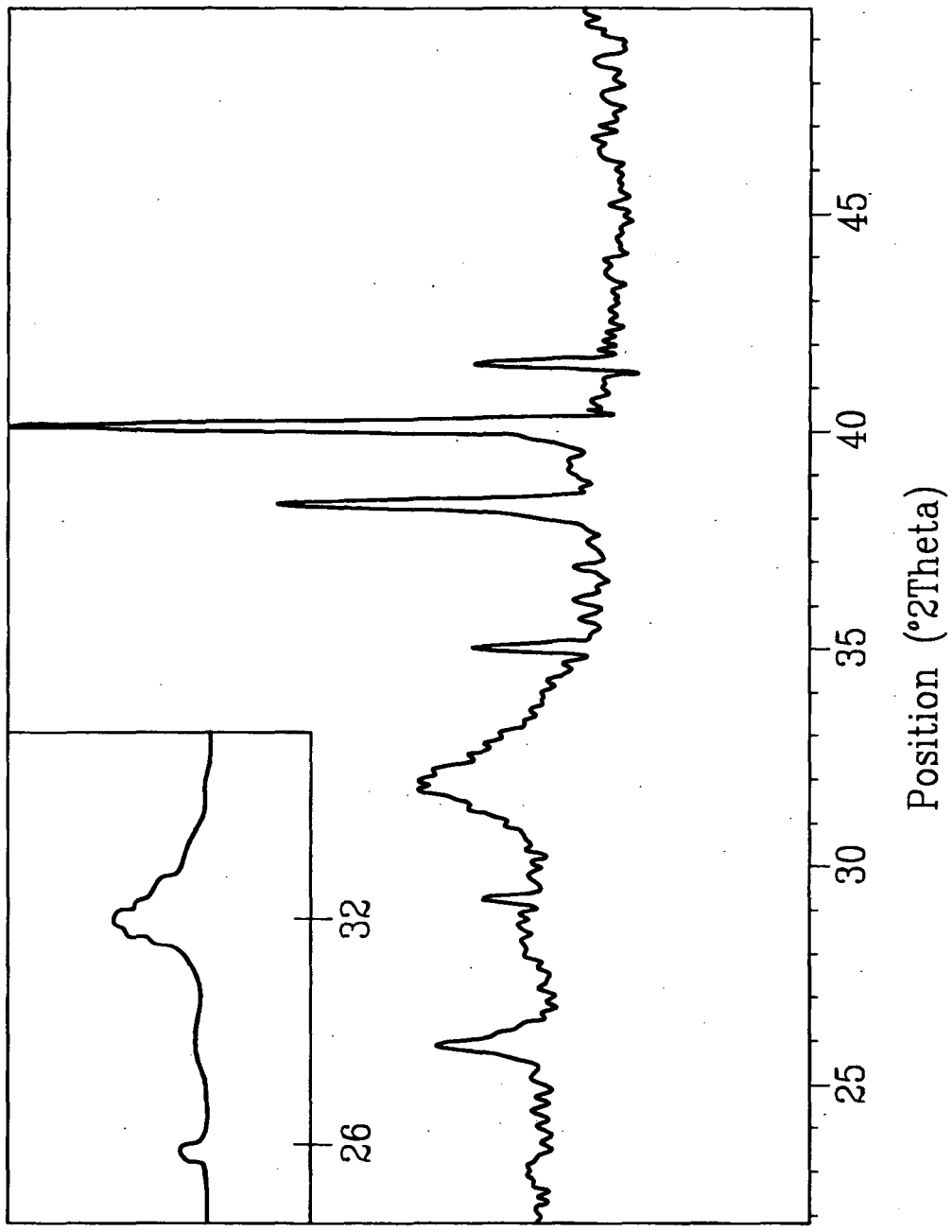


FIG 5



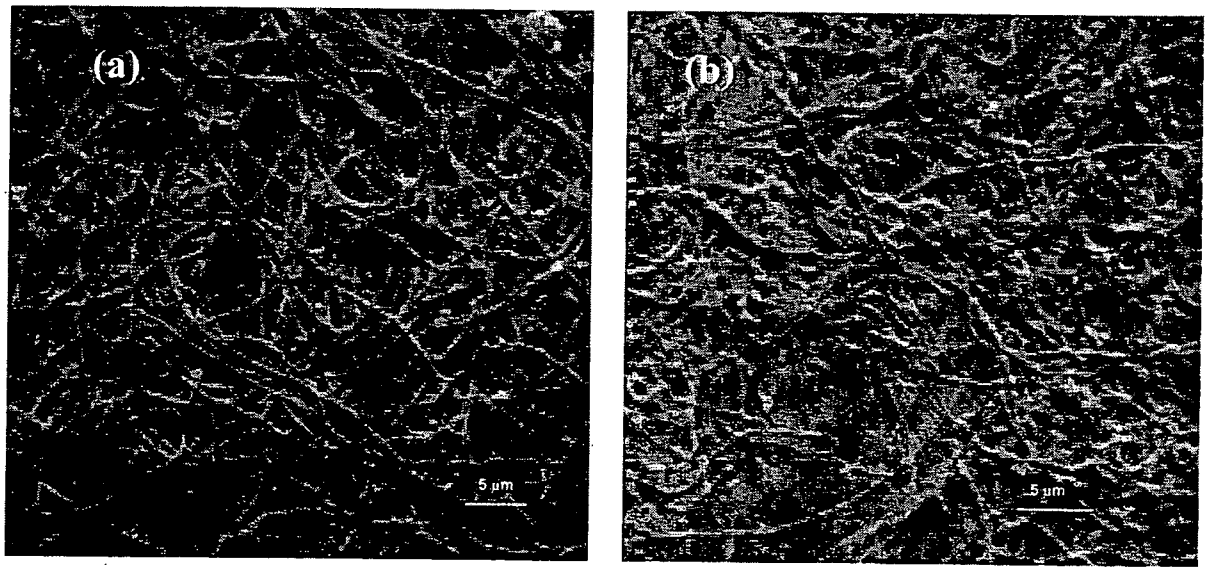


FIG. 6