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# Preparation of Synthetic Hydroxyapatite to Form Biocompatible Coatings on the Implants of Medical Purpose

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## Abstract

A modified technique was proposed for the liquid-phase synthesis of hydroxyapatite allowing one to increase the stoichiometry of the product (the ratio Ca/P = 1.64) in comparison with the standard technique (Ca/P = 1.31). A comparison of the basic physicochemical characteristics for the synthetic and biological hydroxyapatite on the surface of zirconium was carried out. Calcium phosphate biocompatible coatings were obtained on the surface of zirconium basing on the synthesized and biological hydroxyapatite.

**Key words:** hydroxyapatite, synthesis, biocoating, zirconium implant

## INTRODUCTION

In recent decades, in connection with environmental degradation, the number of different diseases demonstrated an increase including those related to the violation of human bone formation processes, with maxillary defects and various dental diseases resulting in the loss of teeth. In order to solve this problem, doctors have been trying to use different metals and alloys, polymers and ceramics [1]. However, in many cases, an organism begins to actively "fight" with the implants, mistaking them for foreign bodies [2].

As it is known, human bone tissue is composed of different calcium phosphates (hydroxyapatite, β-tricalcium phosphate), calcium carbonate and other inorganic and organic compounds [3]. There are many papers published confirming the validity of the choice of certain materials for various medical applications.

The most promising material for use in medicine is presented by hydroxyapatite (HA), basic calcium phosphate $Ca_{10}(PO_4)_6(OH)_2$ [4]. In the 70s of the last century, this substance was found to exhibit a unique biological compatibility, as well as the ability to actively promote the formation and growth of bone cells [5].

Biological HA obtained from natural bones of cattle *via* roasting and grinding is the most accessible and therefore more frequently used [6]. However, such HA has several disadvantages; the main of those is the risk of an infection transmission, potential immunogenicity due to a foreign material [3], as well as heavy metals those were accumulated in the bones throughout the life.

Last time, the most demanded is not HA, but biocomposite materials where the calcium phosphate coating is applied onto the surface of various metal implants. Such coatings are studied at the Institute of Strength Physics and Materials Science of the SB RAS (Tomsk, Russia). Using the method of micro-arc discharge oxidation one can generate bioactive calcium phosphate or oxide coatings on the substrates of titanium [7], zirconium [8] and their alloys, in particular niobium doped zirconium. For medical applications, the most commonly used are titanium and titanium alloys, but the most high strength properties are exhibited by a composite material based on zirconium with a small amount of niobium (1–2.5 mass %) [9].

In connection with the aforementioned, there is a need to replace biological HA by synthesized one which is not inferior with respect to the biological HA in the properties and exhibits a number of advantages including ethical and medical aspects. Obtaining composite «niobium doped zirconium bioactive calcium phosphate coating on the basis of synthetic HA» which in the physical, chemical and mechanical properties is the most close with respect to bone is of current importance, too.

## EXPERIMENTAL

We obtained the HA by precipitation from aqueous solution *via* the reaction [10]

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O$$

For the preparation of stock solutions we used chemical purity grade reagents: calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot 4H_2O$, ammonium dihydrogen phosphate $(NH_4)_2HPO_4$, aqueous ammonia (density $\rho = 0.907$ g/cm$^3$) and distilled water. The synthesis took place under controlled pH level equal to 11–12 with a gradual introduction of a solution $(NH_4)_2HPO_4$ to the aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$.

In the course of the synthesis, we studied an effect of precipitate holding time in the mother liquor on by stoichiometry of the HA obtained, its dispersion level, elemental and phase composition. We determine a practical value of the "solubility product" (equilibrium solubility constant) and the solubility level of HA depending on the medium pH. In addition, we performed a comparative analysis of the properties of the HA powders and the characteristics of the biological HA.

After mixing the initial solutions we performed two series of syntheses. In the first case, the resulting precipitate was to settled for 15 h, then repeatedly washed, centrifuged and subjected to filtration (synthesis No. 1). In the second case, the precipitate was allowed for further aging in the mother liquor during 48 h (synthesis No. 2), and then all of the operations mentioned were performed.

In order to identify the HA obtained we investigated the elemental composition and dispersion level (Philips SEM 515 scanning electron microscope with an attachment for energy dispersion microanalysis). The energy dispersion X-ray analysis of HA samples was performed using Standardless EDAX method based on internal standards of the device. The phase composition was determined on a Shimadzu XRD 6000 X-ray diffractometer using Cu$K_\alpha$ radiation. The registration of X-ray diffraction profiles was carried out with focusing according to Bragg and Brentano. For the phase analysis we used standard ASTM cards.

Using a chemical method, we identified practical values of the equilibrium solubility constant (solubility product) for HA ($K_{sol}$) in water (pH 7) and 0.1 M NaCl solution with the addition of HCl to adjust the medium pH at 298 K. Then, using trilonometric titration, we determined the concentration of $Ca^{2+}$ ions. According to equation

$$Ca_{10}(PO_4)_6(OH)_2 \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^-$$

the expression for the HA equilibrium solubility constant can be presented in the form $K_{sol}(Ca_{10}(PO_4)_6(OH)_2) = [Ca^{2+}]^{10} \cdot [PO_4^{3-}]^6 \cdot [OH^-]^2$

Thus, *via* determining the concentration of calcium ions in the saturated solution, we calculated the corresponding values of the $K_{sol}$ and the negative logarithms of the equilibrium solubility constant p$K_{sol}$.

The HA obtained was used to apply calcium phosphate coating onto the surface of niobium-doped zirconium (1 mass % Nb). The samples for coating (substrates) represented rectangular plates of $10 \times 10 \times 1$ mm$^3$. The coatings were formed *via* micro-arc-discharge oxidation employing MicroArc-3.0 unit [11] in the electrolytes based on 30 % phosphoric acid solution, HA (synthesized or biological) and calcium carbonate [7]. The processing mode was as it follows: voltage 250 V, pulse duration 100 μs, pulse repetition rate 50 Hz, coating deposition time 5 min. The surface morphology of the coatings was studied by means of scanning electron microscopy (Carl Zeiss EVO-50 electron microscope). The roughness ($R_a$) of the surface was measured using a profilometer, model 296 (the State Standard GOST 2789–73). The roughness $R_a$ parameter was determined as a mean deviation of the profile within the basic length:

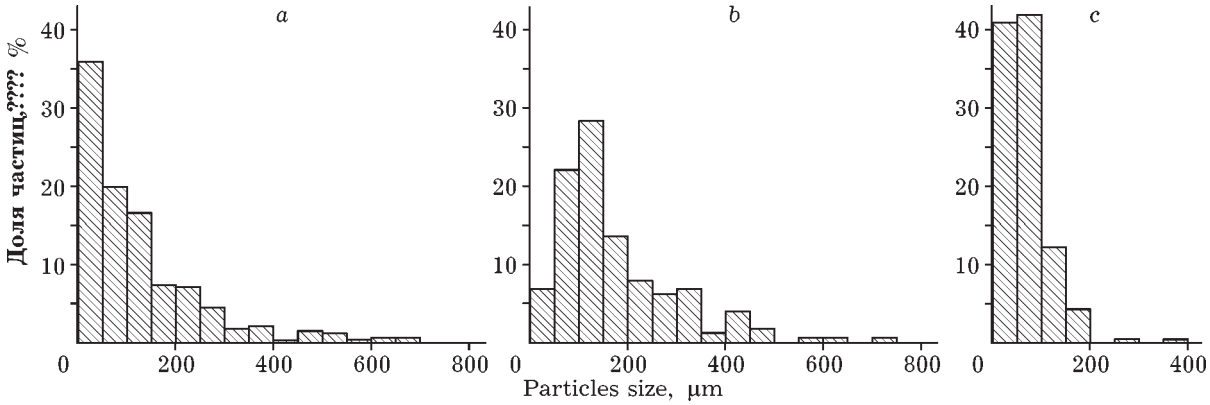


Fig. 1. Histograms of particle size distribution for the samples of hydroxyapatite: *a*, *b* – for the samples obtained in the course of syntheses Nos. 1 and 2, respectively; *c* – for the biological sample.

$$R_a = \frac{1}{L}\int_0^l |y(x)|dx \quad \text{или} \quad R_a = \frac{1}{n}\sum_{i=1}^{n} |y_i|$$

where $x$ is the abscissa of the profile measured along the baseline; $L$ is the basic length; $y(x)$ or $y_i$ is the function describing the profile; $n$ is the number of the partitions of the basic length.

## RESULTS AND DISCUSSION

Figure 1 demonstrates the histograms of particle size distribution for the HA. The particle size was measured using the method of cross-section [12] from the SEM images. In all cases, the size distribution of GA particles exhibited a unimodal character. For the HA obtained from synthesis No. 1, the peak values was localized within the range 0–50 μm.

For the HA with the additional aging of the precipitate (synthesis No. 2) the maximum of the distribution is confined to the range of 100–150 μm. In both cases, there are "tails" elongated in the direction of higher values. For the biological HA, the particle size distribution is located within a narrow range of 0–200 μm, whereas the maximum ranges within 50–100 μm.

One of the main requirements for the HA consists in a proximity to the true stoichiometry of the compound when the atomic ratio Ca/P = 1.67. Figure 2 demonstrates the energy-dispersion X-ray spectra for HA obtained *via* different methods. It is seen that oxygen, phosphorus and calcium are dominated in the composition of HA, no traces of other elements those are not inherent in the HA were found. The elemental analysis data (Table 1) allow us to judge the stoichiometry level of the powders obtained.

The HA resulting from the synthesis with additional settling the precipitate in the mother liquor (synthesis No. 2) exhibits Ca/P ratio equal to 1.64 closest to the stoichiometric ratio value. In the course of synthesis No. 1 a compound was obtained with a deficiency of $Ca^{2+}$ ions and the ratio value Ca/P = 1.31.

By means of the XRD structural analysis, we determined the phase composition of HA

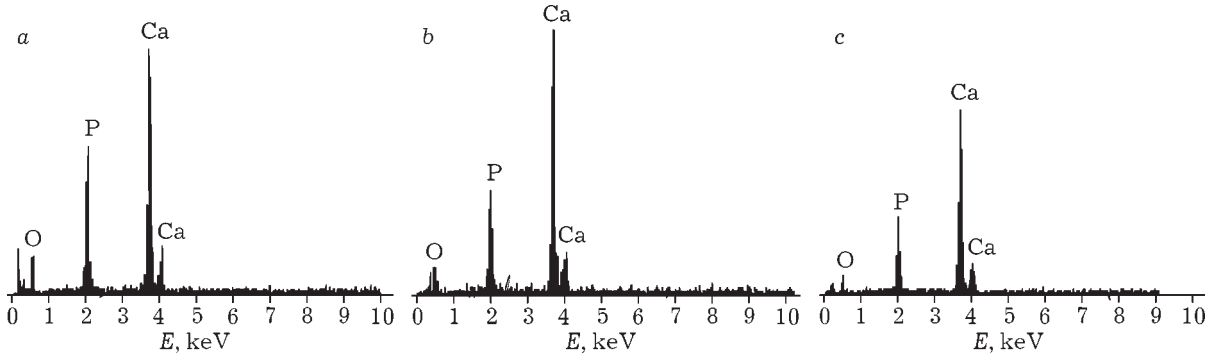


Fig. 2. Energy dispersion X-ray spectra for the samples of hydroxyapatite: *a*, *b* – those obtained from the syntheses Nos. 1 and 2, respectively; *c* – biological HA.

TABLE 1

Results of elemental analysis for HA powders

| Elements | Atomic fraction, % | | |
|---|---|---|---|
| | Synthesis No. 1 | Synthesis No. 2 | Biological HA |
| O | 67.05 | 65.08 | 64.27 |
| P | 14.26 | 13.22 | 13.96 |
| Ca | 18.69 | 21.7 | 21.77 |
| Ca/P | 1.31 | 1.64 | 1.56 |

obtained *via* different methods. Figure 3 demonstrates the XRD profiles of HA powders. We established that in addition to the main phase of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) there is such phase as β-tricalcium phosphate ($Ca_3(PO_4)_2\beta$) present. Its content in the sample of HA obtained without settling in the mother liquor (synthesis number 1) exceeds 50 %, according to quantitative phase analysis data. For the sample of HA obtained in the course of synthesis No. 2, this value amounts to about 4 %. The presence of β-tricalcium phosphate phase in the synthesized powders explains an underscored Ca/P ratio as well as a discrepancy with respect to the stoichiometry of the compound. The structural analysis of biological HA powder demonstrated only the presence of the main phase of $Ca_{10}(PO_4)_6(OH)_2$.

An important factor in the study of the properties of the HA is presented by determining its $K_{sol}$ value. It is known that the solubility of calcium phosphates in water depends on Ca/P ratio: the higher the ratio, the lower the solubility [3]. Table 2 demonstrates the $K_{sol}$ and $pK_{sol}$ for the samples of synthetic and biological HA.

One can see that the lowest value of $K_{sol}$ is inherent in HA obtained *via* additional settling in the mother liquor (synthesis No. 2), and this is in a good agreement with the data from elemental analysis (see Table 1, Ca/P = 1.64). For the biological HA this parameter was higher ($K_{sol} \sim 10^{-36}$), since its Ca/P ratio is lower amounting to 1.56. The highest solubility was observed for HA obtained without settling in the mother

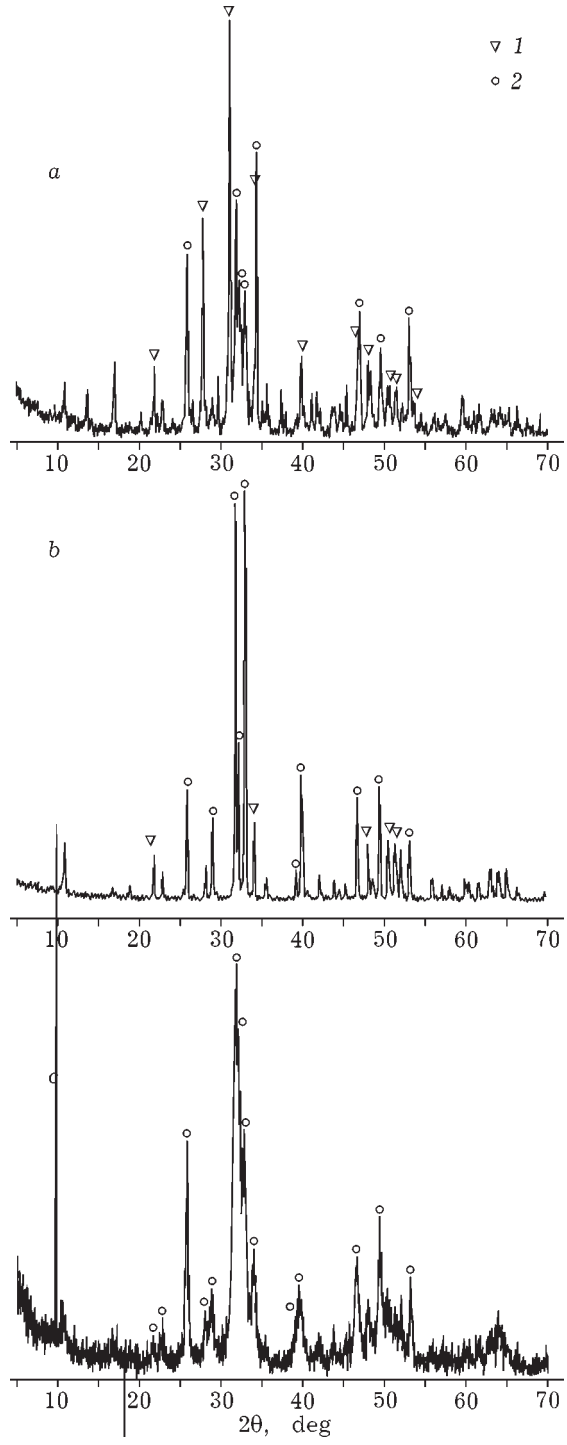


Fig. 3. XRD profiles for the samples of hydroxyapatite obtained *via* synthesis Nos. 1 (*a*) and 2 (*b*), respectively, and for the sample of biological hydroxyapatite (*c*): *1* – $Ca_{10}(PO_4)_6(OH)_2$, *2* – $Ca_3(PO_4)_2\beta$.

TABLE 2

Equilibrium solubility constant ($K_{sol}$) and negative logarithm of equilibrium solubility constant ($pK_{sol}$) for the hydroxyapatite (HA) samples under investigation

| HA samples | $K_{sol}$ | $pK_{sol}$ |
|---|---|---|
| Synthesis No. 1 | $4.174 \cdot 10^{-21}$ | 20.38 |
| Synthesis No. 2 | $9.075 \cdot 10^{-41}$ | 40.04 |
| Biological | $2.870 \cdot 10^{-36}$ | 35.54 |

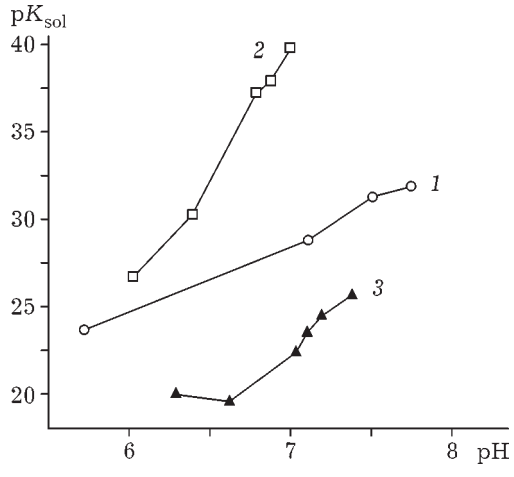


Fig. 4. Equilibrium solubility constant ($pK_{sol}$) of hydroxyapatite depending on pH: *1*, *2* – the samples of hydroxyapatite obtained *via* syntheses Nos. 1 and 2, respectively; *3* – biological hydroxyapatite.

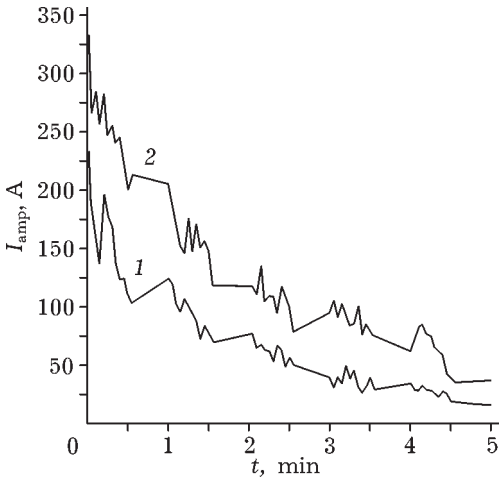


Fig. 5. Amplitude current ($I_{amp}$) strength depending the deposition time ($t$) of calcium phosphate coating onto zirconium in the electrolytes based on biological (*1*) and synthetic (*2*) hydroxyapatite.

liquor (synthesis No. 1), due to a high content of β-tricalcium phosphate phase (50 %).

Figure 4 demonstrates the HA $pK_{sol}$ value depending on the pH of the medium. It can be seen that with decreasing the acidity level of the medium the solubility of all of the HA samples under investigation exhibits an increase. The greatest effect of the medium pH is exerted on the HA obtained in the synthesis No. 2. Thus, one-unit increasing the acidity results in the fact that value of $K_{sol}$ exhibits a 14 orders of magnitude increase (from $10^{-40}$ to $10^{-26}$). However, the solubility of the biological HA depends on the pH of the medium to a lesser extent, that seems to be connected with a higher crystallinity level (roasting the samples of biological HA represents a mandatory part of the process).

Earlier, as a rule, biological HA was used as a component of micro-arc-discharge oxidation electrolyte for forming calcium phosphate coatings on titanium and zirconium [7, 13]. However, in a number of biological properties the biological HA is inferior to a significant extent with respect to the HA sample synthesized with an additional settling in the mother liquor (synthesis No. 2). In this case, the phase and elemental composition of the synthesized HA sample is close to the biological counterpart.

For the objective comparison, the coatings were obtained in the same mode using the electrolytes of the same composition, however, in the first case, the electrolyte was prepared using biological HA, in the second case synthetic HA was used.

Figure 5 demonstrates the amplitude current strength depending on the duration of the deposition of calcium phosphate coating onto the surface of zirconium samples in the electrolytes based on biological and synthetic HA. It can be seen that the initial value of the amplitude current strength for the electrolyte based on biological HA is equal to 330 A, which is almost 100 A higher as compared to that for the electrolyte based on the synthesized HA (240 A). The general view of the curve inherent in the amplitude current strength depending on the duration of the coating deposition for two electrolytes exhibits a similarity: the current strength gradually decreases with the coating deposition time and at the end of the process reaches 35 and 15 A for the electrolytes containing biological and synthetic HA, respectively. From the form of this dependence one can estimate the time of the coating deposition onto the surface of the sample: approaching the curves to a steady-state value indicates the end of the formation of the second dielectric zirconium (calcium phosphate) layer on the surface. Just this time was determined from the experimental curves presented in Fig. 5. With used the electrolyte based both on the biological, and on the synthesized HA, this time amounted to 5 min.

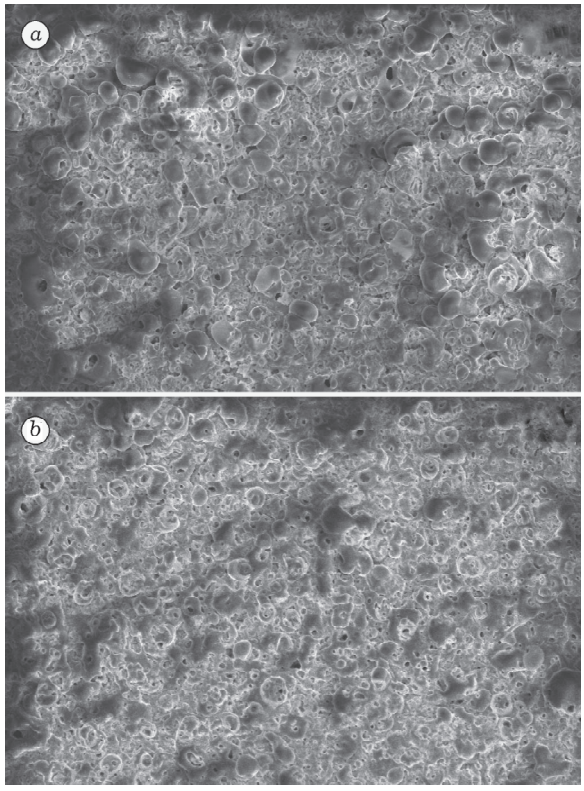


Fig. 6. Calcium phosphate coating on the surface of zirconium obtained in electrolytes based on biological (*a*) and synthetic (*b*) hydroxyapatite.

In the case of using biological HA the coating relief includes areas with the clusters of spherulites formed by a denser sub-layer coating that represents a layer of calcium phosphate "flakes" (Fig. 6, *a*). Spherulites in the electrolyte based on the synthesized HA are less likely formed (see Fig. 6, *b*). To all appearance, these differences in the coating processes are associated with the different nature of the HA affecting its properties (stoichiometry, solubility, dispersion). The coating based on the synthesized HA is characterized by a more uniform surface. The roughness ($R_a$) of such coatings is equal to 3.0–3.5 μm, which is smaller than that for the coatings on the basis of biological HA (4 μm). It should be noted that the roughness is an important factor in the processes of the implant osseointegration into the surrounding tissue. It is known that successful stem cell adhesion to the surface of a coating and their further differentiation produce bone tissue, an optimal value of the roughness ($R_a$) ranges within 2.5–5 μm [7].

## CONCLUSION

Hydroxyapatite was synthesized by means of precipitation from calcium nitrate and ammonium phosphate aqueous solutions. It was demonstrated that holding the precipitate in the mother liquor for 48 h allows one to obtain HA close to the stoichiometric composition both from the elemental and from the phase standpoint. Hydroxyapatite obtained according to the technique proposed could be recommended as the major component of the electrolyte for applying bioactive calcium phosphate coatings onto the surface of valve metals micro-arc oxidation, including zirconium doped with niobium by means of micro-arc-discharge oxidation. The use of the HA synthesized as the major component of the electrolyte provides obtaining biological coatings with the parameters necessary for successful osseointegration of the implant to the surrounding tissues when introduced into an organism.

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