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PULSED ND:YAG LASER PROCESSING OF CALCIUM PHOSPHATE COATINGS ON TITANIUM

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ABSTRACT

Calcium phosphate coatings which are frequently used to improve biocompatibility and osteointegration of the titanium implants are obtained usually by wet chemical or high temperature thermal, plasma processing and suffer from weak titanium/phosphate bonding, absence of efficient process and composition regulation factors. In addition to the known method of increasing the stability of hydroxyapatite coating by creation of gradient Al_2O_3 transitional layer at the titanium surface a new method of pulsed Nd-YAG laser processing of the Ti surface in the presence of hydroxyapatite (HA) and tricalcium phosphate (TCP) was further developed and the results are presented in this paper. Optical and electron microscope investigations as well as EDX revealed that more versatile bioactive coating with regulated micro-heterogeneous calcium phosphate layer may be created this way in comparison with the known deposition methods. Pre-osteoblast cells (HEPM 1486, ATCC) were cultured onto control and covered substrates and showed different morphology.

Keywords: Titanium; Calcium phosphate coating; Laser processing.

INTRODUCTION

Titanium (Ti) is one of the best known material for implants and its biocompatibility is largely determined by the composition and by the macro- and micromorphology of the surface (interface between Ti and bio-tissue) [1-3], which consists of titanium oxides in a simplest case or different calcium phosphate layers, mostly deposited chemically or by the plasma spray method. The surface roughness seems to promote the growth of the bone and strengthening the interconnection between the implant and native tissue [1, 2, 4]. The integration with bone tissue can be improved and accelerated by the presence of different calcium phosphate coatings [1]. Bioactive HA ($Ca_{10}(PO_4)_6(OH)_2$), TCP ($Ca_3(PO_4)$), and other calcium phosphate materials promotes direct attachment to bone tissue [5,6] and have been extensively applied to improve fixation between metal implants and bone tissue [7]. But it is often mentioned that the efficiency and the mechanical stability of the coating is problematic, the solubility of such a cover is not suitable, loosening is observed [1, 8]. Additional investigations are necessary because of the influence of the surface topology and composition, including the titanium oxide layer, on morphogenesis depends on the cell type as well as on the cell adhesion, interaction with other cells and on the bone type [1]. Therefore various and efficient technologies of surface modification must be used to fulfill the requirements of different applications.

Earlier we have developed a new plasma sprayed HA coating on Ti which contained a transitional layer with Al_2O_3 [9] and had better adhesion. Laser fabrication of the surface of

Ti seems to be the most operable and clean (with wide range of temperature, time, ambient variations during the treatment) and can be combined with mechanical or chemical modifications. The creation of transitional layers directly from the deposited HA can be performed by pulsed laser treatment of the Ti surface in the presence of HA [10, 11]. Further development of this new method of pulsed Nd:YAG laser processing of the Ti surface in the presence of the HA and TCP was focused on the structure and composition of the obtained coatings. Investigations of the morphology of growing cells on obtained covers were initiated.

MATERIALS AND METHODS

Flat Ti samples were made by cutting from a commercially available pure titanium (BS2TA2, Titanium International). 2 mm thick samples with 10x10 mm^2 surface were mechanically sandpapered and cleaned ultrasonically in ethanol and distilled water. These samples were used for laser processing with two types of calcium phosphates (CP) simply pressed by a transparent cover glass to the surface or mixed with adhesive and spread on substrates. The first series of initial samples were covered by a layer of hydroxyapatite granules with 40 μm average size, which in turn consist of smaller, 1-2 μm large grains (KERHAP, product of IPM, Kijev, UA). The second series were covered by a layer of TCP grains with 10 μm average size (REANAL, Hungary).
Laser processing of the surface (Fig.1) was carried out in a chamber at normal atmosphere conditions using the 1.06 μm wavelength output of Nd:YAG laser having regulated duration τ and energy of the pulse in 3-5 ms and 2-8 J range respectively. The focused laser pulses interact with the surface in a 2-4 mm^2 area. If a special cylindrical lens is used, the more uniform scanning of larger area. is possible. The sample was placed on a positioning stage (4 in Fig.1), so it was possible to scan the surface with laser pulses with or without overlapping each other and produce large areas covered continuously or by islands of CP (see Fig.2).
Some samples with plasma spray deposited HA were prepared for comparison . The fabrication was performed by 23 kW generator, the standoff distance to the sample was 10 cm, the temperature of the plasma at the surface was 2600 K, the supply of the HA powder to the plasma was 17 g/min. So the Ti surface can be melted at this conditions too, in spite of the compulsory cooling of the back side of the Ti plate.
The surface morphology was investigated by optical microscope (Carl Zeiss, AXIOTEC) as well as by scanning electron microscope (Hitachi S-4300) . Cross-sections of the sample along the path of laser pulses were made to determine the depth profile of the composition and structure. The average composition of the coating was measured by the energy dispersive X-ray analysis (EDX) at the same SEM from the top, the composition profile was measured along the cross-section of the sample.
Human embryonic palatal mesenchymal pre-osteoblast cells (HEPM 1486, ATCC) were cultured in Eagle's minimum essential medium (EMEM) supplemented with Eagle's salts, L-Glutamine, sodium pyruvate (1mM), 10 % fetal bovine serum at 37 ^{0}C and in a 5% CO_2 atmosphere. Cells were cultured onto substrates for 48 hours, fixed in 4 % formaldehyde for 10 min, permeabilized for 10 min. in 0.5% Triton X-100. Cells were dual labeled with FITC-falloidin (Invitrogen, Germany) for F-actin and for the focal adhesion protein vinculin with the monoclonal antibody 7F9 (Millipore) for 45 min at 4 ^{0}C then mounted a glass coverslip onto the substrate surface using Mowiol (Polysciences, USA).The confocal imaging was performed on the inverted confocal laser scanning microscope LSM 510 (Carl Zeiss

Germany) using C- Apochromat 40/1.2 w-corr. water immersion objectives. The size of the images was 512x512 pixels.

RESULTS AND DISCUSSION

The surface of the initial Ti substratum usually consists of 0.5-1 μm deep sporadic scratches and holes due to the sandpaper treatment. The simple Ti surface processed by Nd-YAG laser shuts in the above mentioned regimes may became even more rough at different length scales, since arranged, up to the few μm deep craters with rims can be created by laser shuts, if the delivered energy is above 3.2 J ($P=4,8x10^4$ W/cm^2) in one pulse. The roughening occurs via deformation of the pulse-heated (melted) surface and, possibly, its' additional oxidation enhanced according to the local temperature distribution, as it was supposed also in [12,13]. The temperature of the surface at the moment of irradiation by such a pulse can be increased above 1800 C, i.e. the surface of Ti can be melted ($T_m \cong 1650$ °C). This was supported by the estimations according to the model of laser processing of the metal surface[14] as well as by the presence of surface rafts or even melted droplets at the surface of the single Ti sample treated with pulses of higher intensity. Therefore we can calculate with a surface melting and roughening due to the pulsed YAG-laser treatment of the proper Ti sample even if it is covered by a layer of calcium phosphate with a sufficient transparency at 1.06 μm. The measured transparency of the HA and TCP was up to 60%, depending on the local distribution of the granules on the surface.

The pulsed laser processing of Ti surface, which was preliminary covered by a layer of granular hydroxyapatite or tricalcium phosphate results in a microheterogeneous layer, which has not a sharp interface with Ti, contrary to the plasma sprayed samples (see Fig.3 , a and b) . During the laser pulse the Ti is heated up and the reaction of the calcium phosphate with Ti at the interface occurs. Usually at optimum laser power (surface temperature) HA grains are little "melted in" into the surface due to the laser pulse action and partially decompose, transforms to other structure resulting in a gradient reaction layer between the HA and Ti, which fill in the surface voids. This layer can not be easily removed from the surface, i.e. the mechanical stability is better as in the case of pure plasma sprayed HA coating.

So, the morphological elements of the laser processed calcium phosphate+Ti surface mostly consist of craters and bumps in 1–60 μm range with different compositions. They cover the surface quite homogenously as it is seen in Fig.2, but the space is not filled by HA or TCP only: other phases or even pure Ti are visible between the grains, as it is clear from the cross-section SEM data (see Fig. 4 and Table 1). For this reason the EDX analysis from the top gives the average composition of the cover layer which differs from any calcium phosphate composition.

A mixture of oxides and calcium phosphates with a locally changing depth profile are formed as a result of pulsed laser treatment of the HA-Ti system. In the TCP-Ti system the cover layer seems to be more homogeneous. It may be caused by the smaller thickness of the initial TCP layer at the surface (smaller diameter of the granules) and by the higher stability of TCP. The rapid heating and cooling of the comparatively thick (usually more than 20 μm) multicomponent layer causes local stresses which can lead to the cracks.

The more detailed analysis of the process was done to determine the composition of the resulting covers. It was expected that CP grains can be introduced into the melted Ti without total melting and decomposition. The local EDX analysis of the surface as well as of the cross-sections showed that certain gradient interface layer is created, which starts from the pure HA at the top (if the grains are not completely decomposed, like in Fig.4) and changes towards the pure Ti in a way, presented in Fig.4 and Tabl.1 for a given intensity of laser pulses. EDX data reveals the gradient of Ti, Ca , P and O in the cover layer, which may not contain pure HA or TCP grains at the top if the thickness to pulse energy ratio is small, i.e. the whole initially granular layer undergo changes due to the interaction with molten Ti and decomposition .

Lowering the laser pulse intensity to the optimum results thinner transition layer and the presence of original CP phase at the surface. Furthermore it can be seen in Table 1. that in this layer the Ca: P: Ti: O ratio has a rather well defined value both for HA and TCP. The Ca/P ratio is more than 2 in the case of HA on Ti sample, and little lower in the case of TCP-Ti. The deficit of P may be caused by the high thermal diffusion coefficients of P in titanium at elevated temperatures (10^{-8} cm^2s^{-1} [15]) as well as by the evaporation. The presence of some Ti signal at the top may be caused just by the roughness of the surface and a rather deep volume of analysis. The real structure of these layers, the possible crystalline and amorphous phases need further analysis.

The mechanism of formation of the above reaction layers can be the following: at the irradiation of the original structure (Fig. 5a) first a thin melted Ti and TiO_2 spot forms at the interface (fig.5b) since the melting of Ti is possible under the given surface energy density of the laser pulse. The diffusion and/or convection in this liquid phase can be fast enough to produce the observed structure during such short pulse duration as 10^{-3} s and even to form a continuous mixture layer (fig. 5c.) with decreased P and Ca content. Phase changes and degradation of HA or TCP resulting the rise in CaO content were observed also at high plasma power level during the plasma sprayed HA coating production [13]. Cross sectional analysis of our plasma sprayed Ha-Ti samples (Fig.2.b) also shows the presence of some composition change in ~0.5 μm thick randomly distributed transitional layer , which consists of 21.0 at% Ti, 73.6 at% O, 1.7 at.% P and 3.7 at% Ca that possibly corresponds to titanium oxide with interdiffused Ca and P. Further regulation of this thickness by higher plasma temperatures is not possible because of damage.

Besides the better mechanical properties, adhesion and decreased solubility in body fluids the Ca enriched Ti or Ti oxide layer is interesting because of the known influence on the regulation of bone cell interaction with titanium [16]. Therefore the resulting complex multilayer with wide transitional composition range may be considered as a versatile bioactive coating with parameters, which can be regulated by the composition of the initial interacting components and by the characteristics of the pulsed laser processing as well. First experiments with human embryonic palatal mesenchymal pre-osteoblast cells (HEPM 1486, ATCC) were performed to check the bioactivity of these covers. The morphology of cells is different compared to controls growing on coverslip (Figure 6). The control cells are quite spread and showing an interconnected morphology with structured actin fibers. The cells growing on HA covered Ti surface. became more spindle-like shape with less matured and more dense actin and vinculin structures. Further series of such experiments are under development to clarifie the influence of the cover type on the peculiarities of cell culture formation.

CONCLUSIONS

The pulsed Ng-YAG laser processing of the Ti surface in the presence of calciumphosphates is proposed as a method of formation of heterogeneous, at the micrometer scale, interface layer containing Ca-P compound cover. The enhanced reaction of Ti with HA and TCP creates phases with high mechanical and, possibly, increased chemical stability. The biocompatibility of such implants also seems to be good, since in [15] the presence of Ti in an improved cover layer is evident, as well as in titanium oxide layers on all implants. Our experiments with human embryonic palatal mesenchymal pre-osteoblast cells also showed bioactivity of these covers. The new method is suitable for incorporation of the wide range of other ceramic particles into Ti surface too.

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