HYDROXYAPATITE/ ZRO_2 AND ZRO_2 FILMS FABRICATED BY PULSED LASER DEPOSITION FOR DENTAL IMPLANT APPLICATIONS

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Abstract: The method of pulsed laser deposition was used to fabricate HA and ZrO_2 layers. The depositions of HA and ZrO_2 films were carried out in two different deposition set-ups where KrF and ArF excimer lasers were used. The sample of an implant was a round pad made of titanium alloy Ti6Al4V with the diameter of 10 mm and the thickness of 2 mm. Film properties were characterized by XRD and SEM methods. Ca/P ratio of HA films was studied by WDX method. Scratch test and indentation test were proceeded on ZrO_2 layers. The biological samples were tested of cytotoxicity, attachment and spreading. Also the immunohistochemical reaction was examined.

Introduction

Zirconia (ZrO_2) as a biomaterial has been studied in many papers [1]. Its wide applications are due to its good stability and mechanical properties, especially strength, toughness and high Young's modulus. Hydroxylapatite (HA) is also well known as a biocompatible material and was successfully used to create tooth prostheses [2]. To improve mechanical properties of implants, combination of HA films and ZrO_2 intermediate layers and single ZrO_2 films were created.

Materials and Methods

Zirconia oxide films were created by KrF excimer laser (LUMONICS PM 842) of 248 nm wavelength, frequency 10 Hz and energy 450 mJ. Target was fixed in the distance of 4 cm from the substrate (Fig. 1). Laser energy density on the target was 4 Jcm^{-2}. Layers were fabricated at three substrate temperatures: 20 °C, 400 °C and 700 °C. The ZrO_2 films, which were supposed to be buffer layers for HA/ZrO_2 coatings, were deposited only at room temperature.

HA thin films were created on ZrO_2/Ti6Al4V substrates by ArF excimer laser of 193 nm wavelength, repetition rate 50 Hz and output energy 330 mJ. The trace of the laser beam was 5.3 mm^2. The deposition was proceeded in the H_2O atmosphere at the pressure of 50 Pa. The target was fixed in the distance of 3 cm from the HA substrate and heated up to 600 °C (Fig. 2).

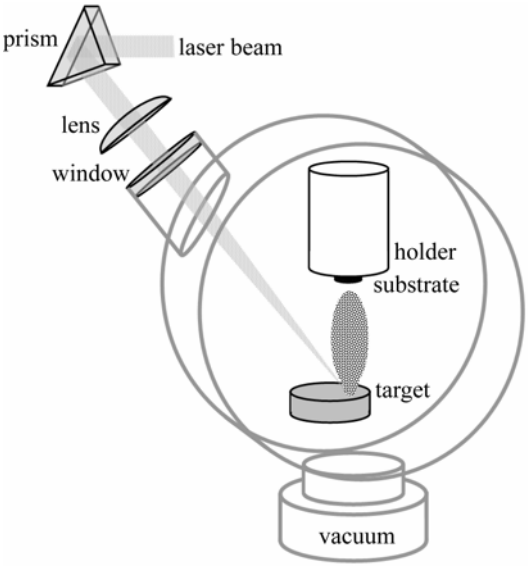

Figure 1: Experimental set-up of ZrO_2 film deposition

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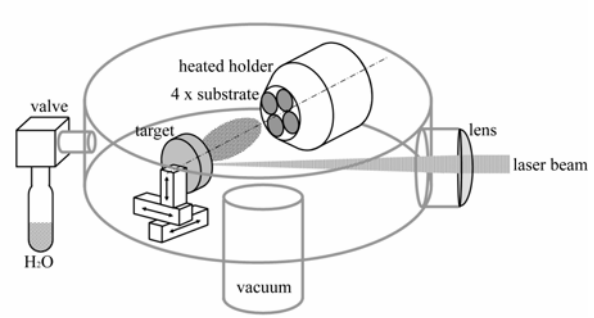

Figure 2: Experimental set-up of HA film deposition

Results and Discussion

Film thickness was measured by mechanical profilometer Alpha Step 500. Film thickness of ZrO_2 buffer layers was 50 nm – 100 nm, thickness of single ZrO_2 layers was 200 nm – 400 nm. HA films were 6 μm – 12 μm thick.

Crystalline structure of the deposited films was characterized by X-ray diffraction analysis (XRD). The XRD analysis proved the presence of crystalline HA in the deposited films (Fig. 3). ZrO_2 films were amorphous.

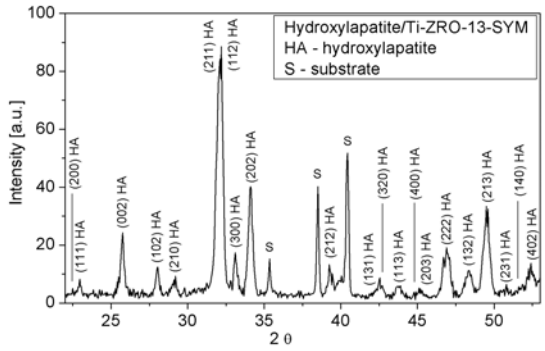

Figure 3: XRD spectrum of HA/ZrO_2 coating

Film morphology was observed by scanning electron microscopy (SEM) (JEOL JXA 733) using 15 kV electron beam and 400x magnification. Smooth surface covered by small droplets was observed for both HA and ZrO_2 layers. Diameters of the droplets were in the range of 5 μm – 20 μm for HA films (Fig. 4) and in the range of 1 μm – 5 μm for single ZrO_2 films (Fig. 5).

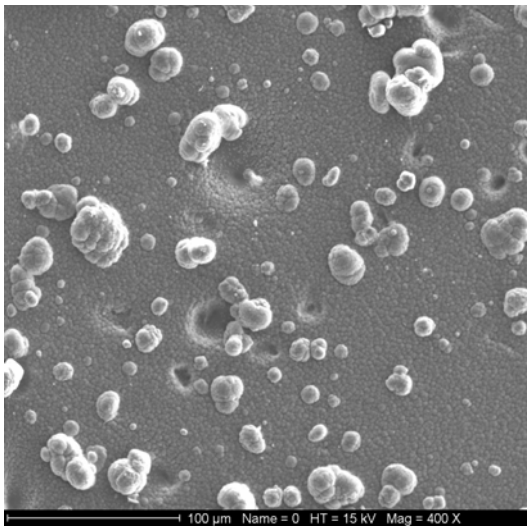

Figure 4: SEM analysis of HA/ZrO_2 film (400x)

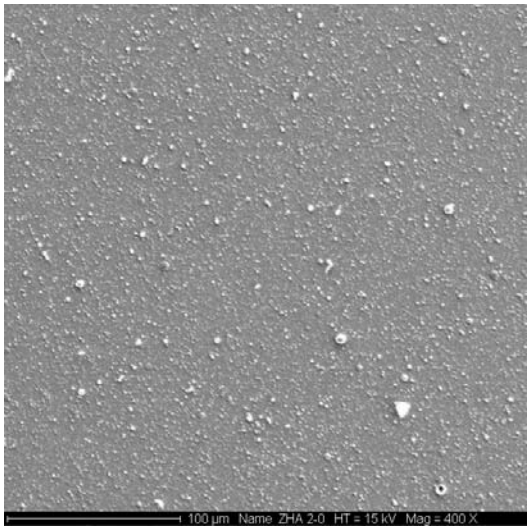

Figure 5: SEM analysis of single ZrO_2 film (400x)

Ca/P ratio of HA films was studied using an electron microprobe and wavelength dispersive X-ray analysis (WDX). The ratio was studied on droplets and on flat surfaces and it differed within the range 2.2 - 2.4 (Table 1). The Ca/P ratio of the droplets was lower. Measuring on larger surface (40 μm x 40 μm), the value of 2.37 was reached.

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Table 1: Ca/P ratio of HA/ZrO_2 films by WDX

Label	A% (P)	A% (Ca)	Ca/P
Droplet	12,41	28,29	2,28
Droplet	12,45	28,20	2,26
Droplet	12,30	28,48	2,32
Droplet	12,23	28,60	2,34
Droplet	12,49	28,14	2,25
Flat surface	11,94	29,10	2,44
Flat surface	11,89	29,19	2,45
Flat surface	12,07	28,87	2,39
Flat surface	11,95	29,09	2,43
Surface 1600 μm^2	12,15	28,74	2,37

Single ZrO_2 films were tested of adhesion by scratch and indentation tests. In the scratch test the load was increasing from zero to 50 N. Critical normal force was in the range 3.0 N – 9.5 N. Maximum force was measured for the samples deposited at 400 °C (Fig. 6). The indentation test was carried out by a Rockwell cone indenter at load of 1470 N. No cracks in ZrO_2 layer were observed for the films created at 20 °C. The films deposited at 700 °C exhibited radial cracks and those created at 400 °C showed concentric circles around the puncture (Fig. 7)

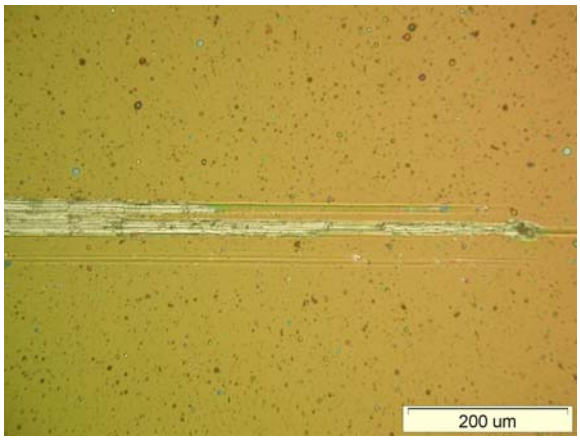

Figure 6: Scratch test of single ZrO_2 film

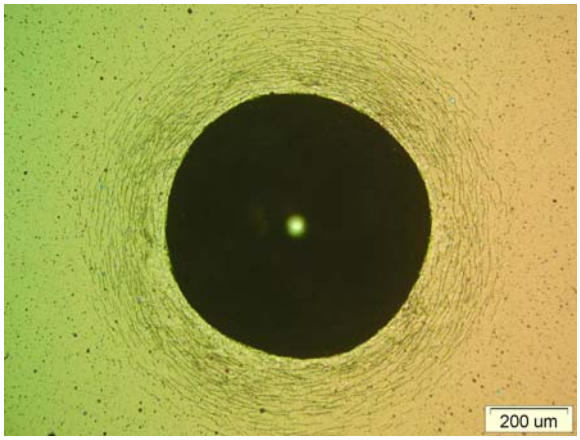

Figure 7: Indentation test of single ZrO_2 film

Several biological tests were provided to analyse HA/ZrO_2 deposited films. The direct test of cytotoxicity was used to evaluate biocompatibility of HA/ZrO_2 samples. Mice line and human fibroblasts were cultivated in the presence of different materials including the studied samples. The Noritake ceramics was used as a positive control and two other materials as a negative control – a sample of red artificial rubber and a sample of dental resin Superpont C+B. The best results were reached for the HA/ZrO_2 samples (Table 2). Cytotoxicity of the HA/ZrO_2 samples was not proved.

Table 2: Test of cytotoxicity of HA/ZrO_2 films

Material	Proportional number of cells [%]	
	3T3 mice fibroblasts	Human fibroblasts
HA/ZrO_2	78	94
Ceramics	65	74
Red rubber	8	0
Resin	43	25

In the test of attachment, amounts of attached fibroblasts onto the surface of the sample and onto the bottom of the dish were numbered in the Bürker counting cella in an optical microscope. Human fibroblasts were placed at a density of 1.105 cells per ml into plates containing the HA/ZrO_2 samples. The cells were cultivated in an incubator at the temperature of 37 °C, at the 3.3% pressure of CO_2 and at 100% humidity in H-MEMd medium. Dishes without samples were used as a control. After 24 hours of cultivation, there were 53% of cells attached onto the surface of the sample and 47% of cells attached onto the bottom of the dish. Total amount of the attached cells fits well with that of control cultivation (Table 3).

In the test of spreading, the human fibroblasts were cultivated for 96 hours. The fibroblasts were placed at the density of 20 000 cells per ml into 1 ml volume of cultivating medium to the HA/ZrO_2 samples. Cultivation proceeded for 96 hours, at the temperature of 37 °C, at the 3.3% pressure of CO_2 and at 100% humidity. Each test has its own control with no samples. There was 47% growth of the cells on the surface of the sample and 53% growth of the cells in the surroundings. The sum of the cells on the sample and in its surroundings is well comparable with the number of cells in the control cultivation (Table 3).

Table 3: Test of attachment and test of spreading of HA/ZrO_2 films

Test	Number of cells (x 1000)		
	Sample surafce	Sample surroundings	Control cultivation
Attachment	58.5	51.5	130.0
Spreading	333.0	378.0	713.0

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Two of the HA/ZrO_2 samples were separated after 72 hours of the cultivation processed in the test of spreading to study the immunohistochemical reaction. The fibroblasts created subconfluent and confluent growth. Films were observed by fluorescence microscope Optihot-2 (Fig. 8).

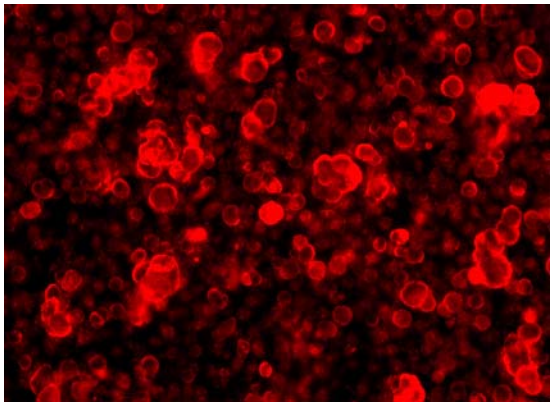

Figure 8: Observation of fibroblast growth on the HA/ZrO_2 films by fluorescence microscope

Conclusions

Thin HA films with intermediate layer of ZrO_2 and single ZrO_2 films were grown by the method of pulsed laser deposition on round samples of titanium alloy Ti6Al4V. All films were well adhesive. Thin films were crystalline or amorphous depending on deposition conditions. XRD analysis proved presence of crystalline HA in the deposited HA/ZrO_2 films. Scanning electron microscopy demonstrated smooth surface covered by droplets with the diameters of 5 μm – 20 μm in case of HA/ZrO_2 films and of 1 μm – 5 μm in case of single ZrO_2 films. The Ca/P ratio of crystalline HA films was higher than that of natural HA, which is 1.68, and it differed within the range of 2.3 – 2.5. Single ZrO_2 films were tested of adhesion. In the scratch test, maximum value of critical normal force was measured for ZrO_2 films deposited at 400 °C. The indentation test showed different reaction of the ZrO_2 samples. The best adhesion was observed for the samples deposited at the temperature of 400 °C.

Biocompatibility of the HA/ZrO_2 films was the best of all tested samples. After 24 hours the test of attachment showed that 53% of cells have attached on the surface of the sample and 47% of cells have attached in the surroundings. The test of spreading showed the 47% growth of the cells on the surface of the sample and the 53% growth of the cells in its surroundings. The fibroblasts created subconfluent and confluent growth.

The perspective of its implantation into the bone is open. Various modifications of the coatings can be used for future research, especially single ZrO_2 coatings are expected to be properly studied for tooth prostheses.

Acknowledgements

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