

COMPARATIVE CHARACTERIZATION OF ARC-PVD ZrN AND PEO HYDROXYAPATITE COATINGS ON PURE Ti-6Al-4V SUBSTRATES FOR BIOMEDICAL APPLICATIONS

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The nanocrystalline films of zirconium nitride have been synthesized using ion-plasma vacuum-arc deposition technique on pure Ti-6Al-4V, whereas the hydroxyapatite coatings were deposited by the method of plasmaelectrolytic oxidation in alkaline electrolytes (hydroxyapatite + 1 M potassium hydroxide). Structure evaluation by X-ray diffraction analysis (XRD), scanning electron microscopy (SEM) with microanalysis (EDS) – were performed to study phase and chemical composition, surface morphology, microstructure of coatings. The formed HA PEO coatings with spheroidal structure contain the phases of CaTiO₃ calcium titanate, CaHPO₄ anhydrous dicalcium phosphate, and Ca₁₀(PO₄)₆(OH)₂ hydroxyapatite. It was revealed that ZrN single-phase coatings of cubic modification with finecrystalline grains of 20 nm in size were formed. This experimental study found that coating Ti-6Al-4V with ZrN and HA positively influences preosteoblast cell adhesion, which results in the almost complete coverage of the surface, along with a less number of cells grown on uncoated titanium surface.

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INTRODUCTION

Orthopedic or dental implants are typically made of titanium or stainless steel and with a wide variety of surface treatments or coatings, which provide sophisticated surface geometries that are needed for implant integration into the bone [1, 2].

One of the most common materials for the manufacture of dental implants is the Ti-6Al-4V alloy [3]. But there are important limitations in the use of titanium/vanadium materials. Because contact of the implant surface with blood, or in the case of dental implants, with dentifrices, can promote degradation of the implant surfaces. As a result, toxic vanadium is released and may be absorbed, thereby contributing to systemic toxicity. Further, progressive deterioration of implant surfaces frequently leads to implant damage, degradation of the bone-implant interface, and ultimately the need for implant replacement. Indeed, ~15% of dental implants fail in the first two years after placement because of surface degradation and consequent failure of the implant-bone interface. These shortcomings necessitate replacement surgery. In addition, implant failure also prolongs total treatment time as the bone tissue around the failed implant needs time to heal prior to revision surgery. Further, failed

implants also lead to the loss of implant-supported restorations, which further drives up re-treatment costs.

Conversely, plasma deposition of coatings creates mechanically and chemically stable coatings on metals and dielectrics that would otherwise be subject to overheating [4]. The application of plasma filters and additional ionization steps enables the production of high quality and consistent adhesive coatings that exhibit excellent protein adhesion and outstanding surface hardness. The application of vacuum-arc ion-plasma deposition for the production of new bone-related implant surfaces could reduce early implant failure and provide unexcelled operating lifetimes and durability for implants.

Hydroxyapatite (HA) Ca₁₀(PO₄)₆(OH)₂ of synthetic origin is an important inorganic biomaterial due to its chemical and structural similarity to the bone tissues, which explains its extensive application in medicine in the form of powders, composites, and coatings [5, 6]. ZrN has been shown to improve titanium implant success by reducing bacterial adhesion and proliferation [7].

In this research we propose to create protective “buffer” layers using coatings produced by vacuum-arc deposition of ZrN onto Ti-6Al-4V alloy to compare with hydroxyapatite and pure titanium alloy.

Examination of tissue responses to implants with these biocompatible coatings with customized nanostructures is a potentially high impact and timely opportunity to enable improved surgical outcomes and implant success.

1. PERIMENTAL SETUP

1.1. VACUUM-ARC DEPOSITION

Zr-N coatings were produced by cathodic arc evaporation process using "Bulat" system (Kharkiv, Ukraine) [8]. A zirconium cathode (99.9%) with a diameter of 64 mm was used. Substrates were made from Ti6Al4V titanium alloy. The substrates were ground and mirror polished to a roughness Ra of approximately 0.02 μm . Then they were washed, chemically cleaned in an ultrasonic bath and dried in warm air. The substrates were placed in a holder at a distance of about 150 mm from the cathode.

In the first step of coating deposition, the chamber was evacuated to a pressure of $2 \cdot 10^{-3}$ Pa. Substrates were ion etched with zirconium ion bombardment by applying a DC bias of -1100 V for 3 min. The arc current was 100 A. The second step was the improvement in the adhesion of the coatings. A pure zirconium layer (about 0.1 μm thick) was deposited on the substrate at the bias voltage of -100 V for 1 min. The third step was deposition of the ZrN coatings at nitrogen pressure of 2 Pa and substrate temperature 450°C. Deposition was performed at substrate bias voltage -100 V. The deposition time was 15 min and produced coatings with the thickness of about 3 μm .

1.2. PLASMA ELECTROLYTIC OXIDATION (PEO) OF HYDROXYAPATITE (HA)

As the main criterion for the formation of synthetic hydroxyapatite, we can choose the necessity of guaranteeing the ratio Ca/P = 1.67 typical of the biological hydroxyapatite. Prior to PEO, the specimen surface was mechanically ground with abrasive paper (SiC) in order to reach a surface roughness Ra = 0.85 μm . The specimens were washed in an ultrasonic bath with distilled water. The composition of electrolytes (pH 12) used for PEO was as follows: 100 g/l Ca₁₀(PO₄)₆(OH)₂ and 56 g/l KOH. We carried out PEO under the conditions of constant voltage (-160 V) with the use of a switching power supply. The duration of oxidation was equal to 1 min. As the cathode, we used a titanium grid.

1.3. XRD, SEM+EDS

The surface topography was studied using JEOL JSM-6390LV scanning electron microscope (SEM) with an accelerating voltage of 20 kV, chemical composition was examined using EDS analysis. X-ray diffraction (XRD) analysis were performed using DRON-3M device, under Cu-K α radiation, monochromated by (002) HOPG in diffracted beam. The XRD line scans were performed in θ -2 θ scanning mode where the incident angle θ and diffracted angle 2 θ are scanned simultaneously.

1.4. OSTEOBLAST CELL GROWTH SET UP

Both sides of the materials (Zirconium nitride, Hydroxyapatite and Titanium alloy) were sterilized by using UV irradiation for 30 min in a laminar flow hood.

The materials were coated with 10 $\mu\text{g}/\text{mL}$ fibronectin (Sigma) in PBS for 30 min at 37°C. After aspirating the coating solution, the materials were air dried in the hood and rinsed with PBS. The materials were placed in a 6-well tissue culture plate (Cellstar) with coated surface faced up. Immortalized mouse calvarial osteoblasts ($1.4 \cdot 10^5/\text{well}$, resuspended in 5 mL complete alpha-MEM, Wisent) were seeded into individual well and incubated in a cell culture incubator for 4 days. The cells were rinsed with pre-warmed PBS, fixed in 4 % paraformaldehyde (in PBS) for 20 min. After (3 \times) washes, the cells were incubated in 100 mM glycine in PBS for 30 min to neutralize PFA. The cells were permeabilized in 0.1 % triton x-100 in PBS for 3 min, and blocked in 1 % BSA/PBS for 30 min. The cells were stained in dark at room temperature with Alexa Flour 488 phalloidin (Invitrogen, 1:500 in 1 % BSA/PBS) for an hour, and counterstained with DAPI (Sigma, 0.0625 M) for 10 min. Photos (10 \times) were taken with a Lecia confocal microscope (SP8). (Pictures were taken randomly from 10 microscopic fields for each material. Two representative pictures from each material are shown).

2. RESULTS AND DISCUSSION

2.1. STRUCTURE, CHEMICAL AND PHASE COMPOSITION

According to the XRD data, the phases of CaTiO₃ calcium titanate, CaHPO₄ anhydrous dicalcium phosphate (monetite), and Ca₁₀(PO₄)₆(OH)₂ hydroxyapatite were revealed on the surface of Ti-6Al-4V after PEO at a voltage of -160 V.

For Zr-N coating, all angles of diffraction peaks with (111), (222), and (220) main reflections were indexed as ZrN phase with a crystal structure of B1 NaCl cubic lattice type (according to card JCPDS 350753, $a = 0.4577$ nm lattice constant). The high intensity of the ZrN (111) Bragg peak, indicates that the ZrN grains grow with the [111] preferred orientation perpendicular to the growth plane.

The surface morphology for HA and ZrN are presented in (Fig. 1). The HA coating is characterized by a spheroidal, porous structure, which is typical of hydroxyapatite (see Fig. 1,a). The surface of ZrN is also typical for PVD method having some craters and a small number of macrodefects identified as drops from the cathode material. The size of macroparticles does not exceed 1 μm (see Fig.1,b).

The SEM mapping for ZrN coating as well as chemical composition analyzed by EDS are shown in Fig. 2. The crystallite size of the deposited ZrN coatings was calculated from the full width at half maximum intensity (β) (FWHM) of the X-ray diffraction angle (θ) of the peak and the wavelength (λ) of copper X-ray radiation using Debye-Scherrer's relation, by taking into consideration that no strains were developed in the coatings: $D = K\lambda/\beta\cos\theta$, where K is a constant ($K = 0.9$ for copper X-ray radiation) and θ – the diffraction angle. Crystallite size of the zirconium nitride coating was 20 nm.

It was shown that at the PEO voltage of up to -160 V, a coating with uniform distribution of pores is formed on

the titanium surface [9]. The Ca content reaches 13.9 at.%, whereas the P content remains practically unchanged. As a result, the Ca/P ratio becomes equal to 1.69, which is close to the value observed for the biological hydroxyapatite (1.67).

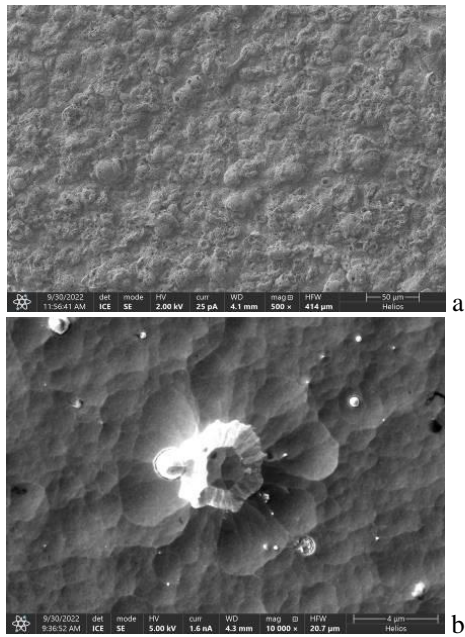


Fig. 1. SEM surface morphology of HA (a) and ZrN (b)

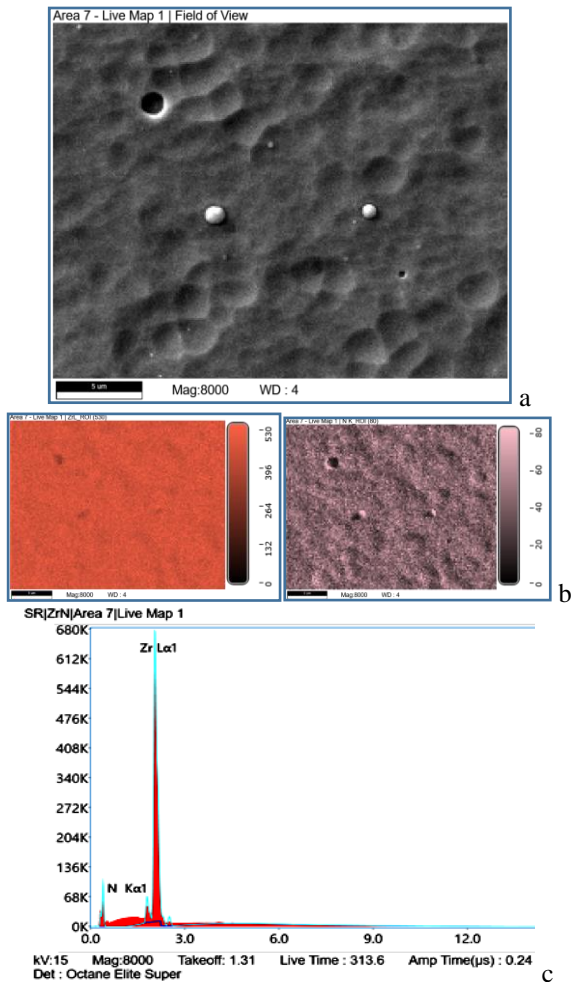


Fig. 2. Surface morphology (a), EDS mapping(b) and EDS spectra (c) of ZrN coating on Ti-6Al-4V

Fig. 3 presents the SEM mapping for HA coating as well as chemical composition analyzed by EDS.

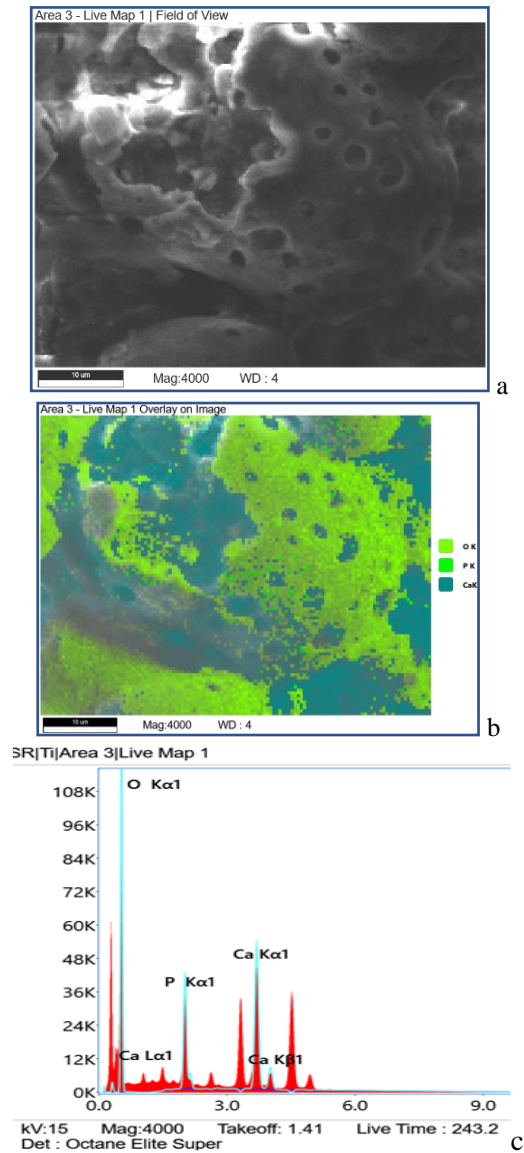


Fig. 3. Surface morphology (a), EDS mapping(b) and EDS spectra (c) of HA coating on Ti-6Al-4V

2.2. OSTEOBLAST CELL GROWTH

To evaluate the cellular behavior on ZrN and HA-coated titanium substrates, mouse preosteoblast cells were seeded onto uncoated and coated specimens, and their adhesion and proliferation were evaluated by cell counting. Fig. 4 presents low power photomicrographs (using epifluorescence imaging of DAPI and phalloidin-stained cells), which are of mouse calvarial osteoblasts cultured on the three materials.

The green dots, which are the nuclei of the mouse calvarial osteoblasts stained with the DNA-binding dye, DAPI, indicate the attachment and the relative cell number of cells that are adherent to the substrate (Fig. 5). As a result, HA coating and ZrN on the surface of Ti-6Al-4V revealed much better osteoblast mouse cell growth and attachment in comparison with pure titanium substrate (see Fig. 5).

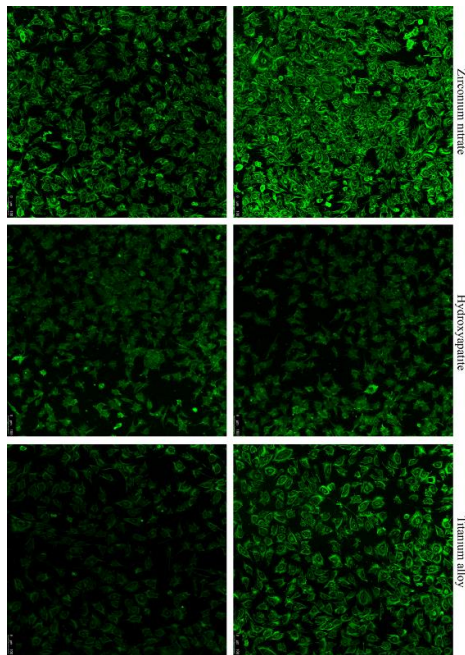


Fig. 4. Low power photomicrographs of mouse calvarial osteoblasts

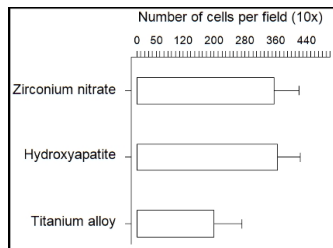


Fig. 5. Comparative results of mouse cell osteoblasts growth

CONCLUSIONS

Zirconium nitride coatings have been synthesized using ion-plasma vacuum-arc deposition technique on pure Ti-6Al-4V substrates. It was revealed that nanocrystalline ZrN single-phase coatings of cubic modification with finecrystalline grains of 20 nm in size were formed.

The formed HA PEO coatings with spheroidal structure contain the phases of CaTiO_3 calcium titanate, CaHPO_4 anhydrous dicalcium phosphate, and $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ hydroxyapatite.

ПОРІВНЯЛЬНА ХАРАКТЕРИСТИКА ПОКРИТТІВ ARC-PVD ZrN ТА PEO ГІДРОКСИПАТИТУ НА Ti-6Al-4V ДЛЯ БІОМЕДИЧНИХ ЗАСТОСУВАНЬ

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Нанокристалічні плівки нітриду цирконію було синтезовано методом іонно-плазмового вакуумно-дугового осадження на підкладки з Ti-6Al-4V, а покриття з гідроксиапатиту наносили методом плазмоелектролітичного оксидування в лужних електролітах (гідроксиапатит + 1 М гідроксид калію). Для вивчення фазового та хімічного складу, морфології поверхні, мікроструктури покриттів проводили структурні дослідження: рентгеноструктурний аналіз (РСА), скануючу електронну мікроскопію (СЕМ) з мікроаналізом (ЕДС). Сформовані покриття HA PEO зі сфероїдальною структурою містять фази титанату кальцію CaTiO_3 , безводного дикальційфосфату CaHPO_4 та гідроксиапатиту $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Виявлено, що утворюються однофазні покриття ZrN кубічної модифікації з дрібнокристалічними зернами розміром 20 нм. Експериментальне дослідження показало, що покриття ZrN і HA на Ti-6Al-4V позитивно впливають на адгезію клітин преостеобластів, що призводить до майже повного покриття поверхні, порівнянно із меншою кількістю клітин, вирощених на титановій поверхні без покриття.

This experimental study found that ZrN coating on Ti-6Al-4V and Ti-6Al-4V/HA positively influences preosteoblast cell adhesion, showing almost similar values of as grown cells covering the surface, along with a more evenly spread appearance compared with cells grown on uncoated titanium surface.

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