Wettability Engineering and Bioactivation of Hydroxyapatite Nanoceramics

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ABSTRACT

The major trends in biotechnology and tissue engineering are development of materials with controlled surface substrates. One of the critical factors influencing adhesion of biocells is wettability properties of the biomimetic materials. Hydrophilicity/hydrophobicity of any solid state surface depends on interfacial physical properties which can be varied by topographic nanostructuring, surface chemical modification and external electric field.

We report about new method enabling reversible switching or gradual transition of wettability properties of materials by surface potential modulation. The use of low energy electron irradiation allows achieving tunable wettability of the hydroxyapatite in a wide range of contact angles from 10° to 100° with accuracy of ±3°. Tailoring the gradually varied wettability state in the hydroxyapatite nanoceramics enabled the differential binding of various biological cells as well adhesion of various sorts of bacteria demonstrating their selective immobilization.

Keywords: wettability modification, low energy electron irradiation, wettability patterning, hydroxyapatite nanoceramics and implant coating, bioactivation, biocells adhesion.

1 INTRODUCTION

Modification of solid surfaces wettability is intensively studied for understanding fine mechanisms of biological cell integration and development of biomimetic materials for tissue engineering, drug and gene delivery, *etc* [1]. The adhesion of biological macromolecules such as proteins and DNA on biomaterial surfaces is mainly attributed to hydrophobic/hydrophilic properties of the biological substrates, and wettability is a critical factor in biological cells immobilization [2].

Recently, it has been possible to change surface wettability by the use of diverse techniques on the basis of extrinsic surface modification [1] including deposition of self-assembled monolayers (SAMs), electrical, light-induced and electrochemical methods, as well by changing the environmental conditions. Among the surface modification methods, the formation of SAMs proved to be a simple and practical technique for controlling wettability and biological adhesion [3]. However, aforementioned techniques are accompanied by either surface chemical reactions or form foreign molecules structure on the modified surfaces.

One of the widely used biomimetic materials developed for replacing defective bone tissues [4] and immobilization of various biocells [5] is hydroxyapatite (HAp). Recently applied nanotechnology has allowed fabricating the HAp coatings with particles 15-20 nm for high-strength orthopedic and dental composite [6]. A great advantage of the HAp is beneficial biocompatibility for bones regeneration and formation of new bone tissue on their surface [4].

In this paper we report on a new method of wettability modification in a wide range of contact angles of solid state materials by surface potential modulation. The method applied to the HAp ceramics and HAp coated human implant enables to strengthen their bioactivity and adhesion of biocells of different origin.

2 HYDROXYAPATITE CERAMICS FABRICATION AND CHARACTERIZATION

HAp ($Ca_{10}(PO_4)_6(OH)_2$)) is primary mineral ingredient of bones, tooth and calcified tissues. Developed over the past dozen of years as implantable material it demonstrates high biocompatibility and osteoconductivity for bones regeneration and formation of new bone tissue on their surface.

HAp nanopowder was fabricated by several steps using both fine mechanic treatment and chemical reactions. Mechanical activation was performed under an air environment in a planetary mill containing two steel drums and steel balls. Transmission Electron Microscopy analysis showed that the size of powder particles was about 20-100 nm. Particles of typical 40 nm size were extracted for the ceramics manufacturing and used as a raw material for preparation of ceramic platelets. The HAp powder was annealed at 1100 ºC in two hours and then dispersed in alcohol in two minutes. The platelet-like samples (height 2-3 mm, diameter 5 mm) were fabricated using dry pressing HAp powders (weight 0.1 gr). A press form greased by rapeseed oil was used for two stage compaction. Pressure of 250 MPa and 375 MPa during the first stage and second one was applied.

High resolution X-Ray Photoelectron Spectroscopy (XPS) and time-of-flight secondary-ion mass spectrometry (TOF-SIMS) analysis were applied to characterize a chemical composition of the titanium implant with HAp coating. The XPS measurements were performed in Ultra High Vacuum ($3\cdot10^{-10}$ Torr pressure) using 5600 Multi-Technique System (PHI, USA). The samples were irradiated with a monochromatic Al Kα source (1486.6 eV). The TOF-SIMS analysis was carried out using a Physical Electronics TRIFT II TOF-SIMS instrument using a 15 kV Ga^+ primary ion gun. Topography features were observed by Atomic Force Microscopy (AFM) (Multimode; Digital Instruments) in tapping mode and were also imaged by Scanning Electron Microscopy (SEM) using a Raith 150 Ultra High Resolution E-Beam Tool (Raith; GmbH Germany). The DC conductivity measurements were conducted by HP-4339 High Resistance Meter in conjunction with a HP-4284 Precision LCR Meter.

AFM and SEM topography studies of the HAp ceramics showed identical topographic features. Statistical analysis gave the average size of ceramic grains around 300 nm and dispersion of 100 nm. The porosity of the fabricated samples characterized by the use of scanning probe microscopy software was around 20 %. DC conductivity measurements showed the value around 10^{-8} 1/Ω·cm. Composition and atomic concentrations of the elements contained in the investigated ceramics was determined from XPS and TOF-SIMS measurements. A typical formula for HAp is $Ca_{10-x}(PO_4)_{6-x}(OH)_{2-x}$, where X ranges from 0 to 2, giving a Ca/P atomic ratio of between 1.67 and 1.33. The Ca/P molar ratio of studied ceramics obtained from XPS measurements was found 1.31 and was related to low stoichiometry composition.

Wettability state was estimated by measuring contact angles by sessile drop of distil water deposited on a sample surface. The measurements were performed by Olympus MX-50 microscope combining CCD color camera. Influence of heterogeneity (chemical or physical) of studied sample surfaces was examined by contact angle hysteresis measurements using the tilting plate technique. This method uses a liquid droplet that is placed on a sample surface. The latter is tilted at a progressively higher angle until the droplet starts to move that allows the determination of advancing and receding contact angles. The wettability studies were carried out at a temperature of 26±1 °C and at a humidity of 45±5 % RH.

3 TRAPS STATES SPECTROSCOPY IN HYDROXYAPATITE NANO-BIO-CERAMICS

In this part of the work the results of electron states spectroscopy of the HAp are presented [7] using photoluminescence (PL) and surface photovoltage spectroscopy (SPS) as well thermostimulated exoelectron emission and thermoluminescence methods [8] for studies of electron energy spectrum**.** Optical absorption spectra were measured with a Genesis-5 spectrophotometer (Milton Roy, USA) equipped with PC-IBM. PL excitation and emission spectra were measured with a FP-6200 (Jasco, Japan) spectrofluorometer supported by a Pentium 4 computer. The signal-to-noise ratio of the instrument is around 450:1. The wavelength range provided by the FP-6200 is 200 nm to 800 nm (excitation) and 200 nm to 900 nm (emission) with the WRE-362 red sensitive photomultiplier. Appropriate Long Pass and Cut Off optical filters were applied in order to exclude stray light and second-order effects. SPS studies are based on the Kelvin probe technique [9] which measures the contact potential difference (CPD) between a vibrating reference probe and sample surface subjected to a light illumination. The obtained photo-induced variation of ΔCPD spectrum contains information about semiconductor type of conductivity, electron affinity, band gap local states and built-in potentials. It should be noted that a great advantage of SPS compared to PL optical method is an opportunity to distinguish between electron and hole traps by estimation of absolute position of a localized state [10]. The found experimental conditions and developed setup allowed studying electron states spectroscopy both in the bulk and near the surface layer in the region 20-400 Å as well to distinguish surface and bulk states. The conducted studies allowed estimation of energy positions of bulk and surface electron (hole) states as well energy gap in the HAp ceramics. The value of the energy gap in the HAp was found as E_g=4 eV. The found energy of six localized states is in the range 2.6-3.3 eV. The studies of electronic properties of the HAp ceramics showed that the HAp ceramics represents a P-type wide band gap semiconductor (Figure 1) with original positive band bending.

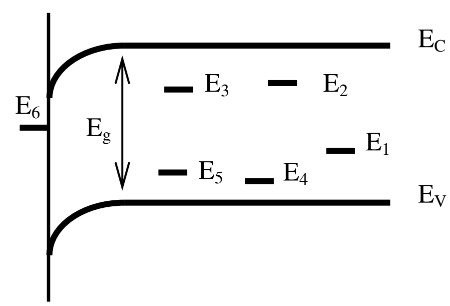

Figure 1: Band diagram of hydroxyapatite ceramics, where E_1-E_4 and E_5 are the bulk states, E_6 are the surface state and E_g is the band gap energy.

4 WETTABILITY ENGINEERING AND PATTERNING OF HYDROXYAPATITE CERAMICS

Among various types of surface interactions the origin of proteins adhesion on biomaterial surface in orthopedic implants and engineered tissues is mainly ascribed to wettability of the biological substrates considering hydrophobic/hydrophilic properties as a leading mechanism responsible for the cells immobilization. Diverse surface modification techniques have been found to permanently alter this basic material property. All of them are usually accompanied by surface chemical reactions and some of these techniques allow local variation of wettability [1].

We found that variation of the population of the surface states and the bulk states near the surface in the HAp ceramics induced by low electron energy irradiation leads to variation of a surface potential (Figure 2) resulting in wettability modification of this biomimetic material [11]. That allows fabricating both homogenous distribution of wettability (Figure 3) and wettability patterning of the HAp. The method enables to tailor the high resolution HAp wettability patterning, which opens the avenue for new generation of biochips, based on arrayed HAp, biocells immobilization, gene transfer, *etc*. Variation of the HAp wettability was done by the use the electron irradiation system employed the Kimball Physics electron gun. The treatment was performed in vacuum 10^{-7} Torr using electron flux low electron energy flux, current density 100 nA/cm^2. Wettability patterning was tailored by exposing the HAp samples to local electron irradiation through specifically designed Si-mask. The mask contained arrays of circle-shaped holes of 200 μm diameter and period of 400 μm (Figure 4).

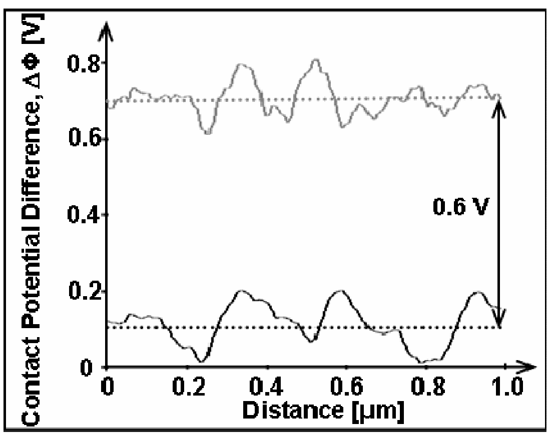

Figure 2: Contact (surface) potential difference profile measurements of hydrophilic (black line) and hydrophobic (grey line) states of the hydroxyapatite surface obtained from Kelvin Probe Force Microscopy measurements.

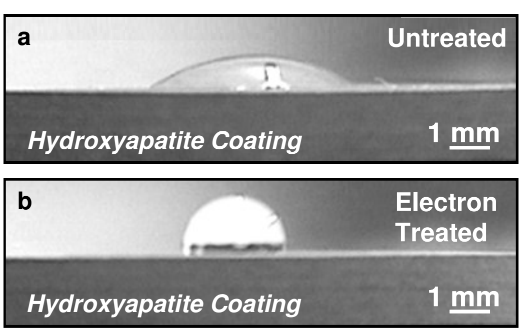

Figure 3: Hydrophilic (a) and hydrophobic (b) states of the hydroxyapatite sample fabricated by low energy electron irradiation.

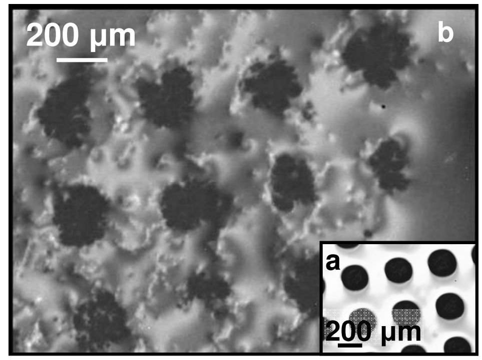

Figure 4: Optical images of Si mask (a) and wettability patterning on the hydroxyapatite surface (b).

5 SELECTIVE ADHESIONS OF BIOCELLS ON FEMORAL IMPLANT WITH HYDROXYAPATITE COATING

One of the critical problems in the field of medical implants is development of biomaterials inherently resistant to bacterial adhesion and growth [12]. Bacteria may adhere and colonies the material either by contamination during surgery or by seeding from a distant site through the blood circulation [13]. Such infections often may result in failure of the implant due to localized bone destruction [14]. Analysis of numerous publications presented in the review paper [15] shows that there is no commonly accepted mechanism of bacterial adhesion. The adhesion process involves two interaction agents, the live bacteria and biomaterial substrate, where each side possesses its own intrinsic features. The basic physico-chemical properties of these two agents should be adjusted to each other to strengthen or impair the adhesion. For example, it has been shown that hydrophilic materials possess much higher resistance to bacterial adhesion than those with hydrophobic properties. Among various biological and physical properties influencing bacteria adhesion, two factors were chosen as leading mechanism in their immobilization: bacteria's wettability and electric charge. The hydrophobicity/hydrophilicity of bacteria varies according to bacterial species and is influenced by growth medium, bacteria age and bacterial surface structure [16].

In this chapter of the paper we report on gradual tuning of the surface wettability in the commercially available titanium femoral implant coated by the HAp layer that allows fabricating homogenous distribution of desired wettability state on the HAp surface in a wide range of contact angles from θ=10˚ to θ=100˚ (Table). It was found that hysteresis between the advancing and receding contact angles was 3±2˚ for hydrophilic state (θ=10°) and 27±3° when the HAp surface was modified to the hydrophobic state (θ=100°). Selective immobilization of various bacteria such as Escherichia coli, Pseudomonas putida and Bacillus subtilis are demonstrated in the Table.

Contact Angle, θ	Escherichia coli	Pseudomonas putida	Bacillus subtilis
θ=20˚	±	-	-
θ=30˚	+	-	-
θ=40˚	±	-	-
θ=60˚	-	-	-
θ=80˚	-	+	±
θ=100˚	-	±	+

Table: Adhesion of various bacteria on the hydroxyapatite surface as a function of wettability modulation (θ is the contact angle). +, -, ± bacterial adhesion and not adhesion and intermediate reaction.

It should be noted that developed low energy electron irradiation method leads to the reversible wettability modification of the orthopedic implants with the HAp coating without generating bulk and surface defects or modifying surface topography and phase state of the material. Tailoring any wettability state in a wide range of contact angles by variation of injected and trapped electron charge enables selective adhesion of various bacteria on the HAp surface. The developed method of surface wettability allows fabricating different susceptibilities of the HAp surface to infection, because adhesion and growth of infecting bacteria may be controlled by the HAp surface properties, like hydrophobicity.

Series of the HAp samples were also electron irradiated using increasing exposure time resulting in wettability tailoring in the range of θ=10˚ to θ=100˚. The differences in wettability resulted in differential binding of biological molecules, in correlation to their level of hydrophobicity. DNA, a very hydrophilic molecules due to the phosphate groups in the sugar-phosphate backbone, bound preferentially to the high wettability surface (θ<50˚). In contrast, the binding of BSA protein which contains hydrophobic domains was more pronounced at low wettability (θ>50˚).

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