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Tunable hydroxyapatite wettability: Effect on adhesion of biological molecules

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Abstract

Surface wettability modifications of biocompatible materials and wettability patterning are attractive methods for directed biological cells immobilization for tissue engineering, drug delivery, gene transfer, etc. Hydroxyapatite is known as an implantable biomimetic material and a substrate for effective adhesion of biological cells of various origins. Here we report the use of a low-energy electron irradiation to achieve tunable wettability of the hydroxyapatite in a wide range of contact angles, from 10° to 100°, with accuracy of ±3°. The incident electrons generate electron/hole pairs resulting in significant variation of the surface potential of the hydroxyapatite semiconductor and give rise to pronounced wettability modification. Tailoring the gradually varied wettability state in the hydroxyapatite nanoceramics enabled the differential binding of biological materials with different surface properties, such as bovine serum albumin (BSA) and deoxyribonucleic acid (DNA).

Keywords: Surface modification; Wettability; Hydroxyapatite; Adhesion molecule

1. Introduction

Controlling 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. [1–3]. The adhesion of biological macromolecules such as proteins and deoxyribonucleic acid (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 [2,4].

Recently, it has been possible to change surface wettability by the use of diverse techniques on the basis of extrinsic surface modification including deposition of self-assembled monolayers (SAMs) [2,3], electrical [5], light-induced [6] and electrochemical methods [7], as well by changing the environmental conditions with various solvents [8], temperature [9], pH [10] and surface pressure [11]. Among the surface modification methods, the formation of SAMs proved to be a simple and practical technique for controlling wettability [2,6] and biological adhesion [3,12]. However, the aforementioned techniques are accompanied by either surface chemical reactions or form foreign molecules structure on the modified surfaces.

Among the various biocompatible substrate materials, hydroxyapatite (HAp) is widely used as an implantable bioactive agent that replaces defective bone tissues and also serves as a substrate for effective adhesion of various biological cells. Wettability plays the critical role for all these uses [13,14].

Here we present an alternative approach for gradual tuning and on/off switching of the surface wettability of the HAp, induced by a low-electron energy irradiation [15]. The electron/hole charges, generated by the electron beam, are trapped in the vicinity of the HAp surface, and resulting in significant wettability tuning without applying external electric field. This method allows the fabrication of homogenous distribution or patterning of the wettability state on the HAp surface. The differential immobilization of biological materials, such as bovine serum albumin (BSA) and DNA on the wettability-engineered HAp ceramics surface is demonstrated.

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2. Materials and methods

The HAp nanopowder was fabricated by several steps using fine mechanical treatment and chemical reactions, $3Ca(H_2PO_4)_2{\cdot}H_2O + 7Ca(OH)_2 = Ca_{10}(PO_4)_6(OH)_2 + 15H_2O$. Mechanical activation was performed under air environment in a planetary mill containing two steel drums and steel balls. The nanocomposite particles were tested by means of X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FT-IR) and the specific surface analysis. Sizes of particles were defined by means of transmission electron microscopy. The obtained data shows, that all probes are enough crystallized HAp in the form of nanoparticles with sizes in range from 20 up to 100 nm. The HAp particles of typical 40 nm size were extracted from nanopowder mass and used as a raw material for preparation of the ceramic samples. The platelet-like samples (height of 2 mm; diameter of 5 mm) were fabricated using dry pressing HAp powders. A press form greased by rape seed oil was used for two-stage compaction. Pressure of 250 and 375 MPa during the first stage and second one was applied. After pressing the resulting ceramic bodies were sintered with heating rate of 5 °C/min to 1100 °C annealing at that temperature for 60 min. Sintered platelets were cooled down to room temperature within an oven. The XRD analysis and FT-IR spectra of the synthesized samples show the good formed crystalline structure of the HAp. A typical formula of the HAp is $Ca_{10-X}(HPO_4)_X(PO_4)_{6-X}(OH)_{2-X}$, where X ranges from 0 to 2, giving a Ca/P atomic ratio of between 1.33 ($X = 2$) and 1.67 ($X = 0$) [16]. The composition and atomic concentrations of the elements contained in the investigated HAp ceramics were determined from X-ray photoelectron spectroscopy (XPS) measurements. The Ca/P molar ratio obtained from atomic concentration measurements was found to be 1.66, which is the evidence that the fabricated HAp ceramics is close to the stoichiometric composition.

Topography features were studied by atomic force microscopy (AFM) (Multimode, Digital Instruments, USA) in tapping mode. Additionally, the HAp samples were imaged by conventional scanning electron microscopy (SEM) using a Raith 150 ultra high-resolution e-beam tool (Raith, GmbH Germany). The roughness and the porosity analysis were performed using the scanning probe microscopy software.

The low-energy electron irradiation was used to achieve tunable wettability of the HAp in a wide range of contact angles. The electron charging of the studied HAp samples was performed using a commercially available electron gun (EFG-7, Kimball Physics Inc., USA) in vacuum 10^{-7} Torr with constant excitation energy of $E_e = 100$ eV and electron current density of $J_e = 100$ nA/cm^2. The exposure time was varied in the range of $t = 0$–3000 s in accordance to desire surface wettability state.

The HAp surface properties modifications were detected by measurements of a surface electric potential and wettability variation. The XPS and AFM were used to control electrochemical and topographical surface modification induced by a low-energy electron beam. The surface electric potential distribution was studied by the Kelvin probe force microscopy (KPFM) [17]. The method is based on measuring the contact potential difference between a reference electrode with invariable work function (Si-nanometer-size tip with Ti–Pt coating) and a studied sample surface, using modified AFM. The instrument has a high resolution for both the surface potential, $\Delta\Phi$ (better than 5 mV) and the lateral dimension (<30 nm) and allows the simultaneous imaging of topography and surface potential difference.

The surface hydrophobicity was studied by measuring contact angles with a sessile drop of distilled water deposited on a sample surface. The optical wettability inspection was performed by an inspection microscope (Olympus MX-50, Opelco, USA) combining charge-coupled device camera and digital imaging techniques. The measured contact angle characterizes not only the intrinsic wettability of a solid surface, but also reflects the contribution of the surface roughness and chemical heterogeneity of the observed wettability state [18]. The tilting plate technique measures the left and right sides of a drop when the surface under study is tilted with respect to the optical axis. Therefore, fluid accumulates in the leading (advancing) edge of the drop and drains from the trailing (receding) edge. The difference between advancing receding angles are attained when the drop is tilted to "the point of incipient motion" and the drop is free to move. The hysteresis effect may be absent or negligible in some systems, but in most systems, it is appreciable [19]. The volume of the liquid was kept constant (2 μl) all over the contact angle measurements of different specimens. The wettability investigations were carried out with an accuracy of ±1° at a temperature of 26 ± 1 °C and a humidity of 45 ± 5% RH.

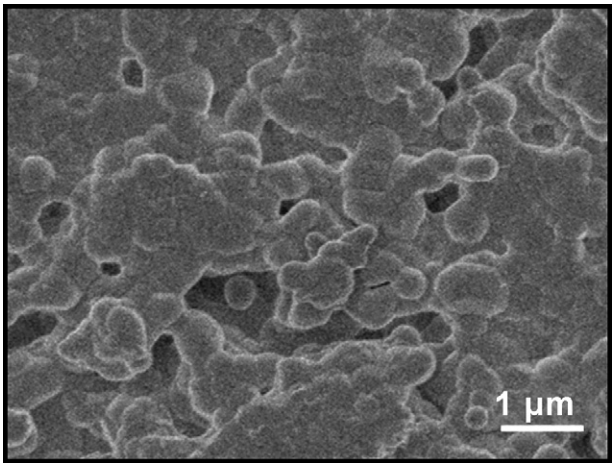

Fig. 1. Scanning electron microscopy image of the hydroxyapatite ceramics, magnification of 20,000×.

Biological materials with different surface properties, such as BSA and DNA, were used to confirm the wettability modification of the treated HAp surface. BSA and single stranded salmon sperm DNA (Sigma–Aldrich Corporation, St. Louis, MO, USA) were dissolved in distilled water at a concentration of 1 mg/ml and applied to the treated HAp ceramic surface (the volume of the solution was 100 ml). Since the DNA and BSA concentration was quiet low the pH was that of the water (~5.5). The samples were incubated 15 min at room temperature with no vibrations, washed several times with distilled water and then Giemsa stained (Sigma–Aldrich Corporation) to visualize the biomolecules.

3. Results

Figs. 1 and 2 illustrate SEM and AFM surface topography images of the studied HAp ceramics, respectively. The SEM and AFM images of the HAp are presented at approximately the same lateral magnification. The two images show similar surface structure, however, they differ in the other types of information that can be acquired on this sample. The three

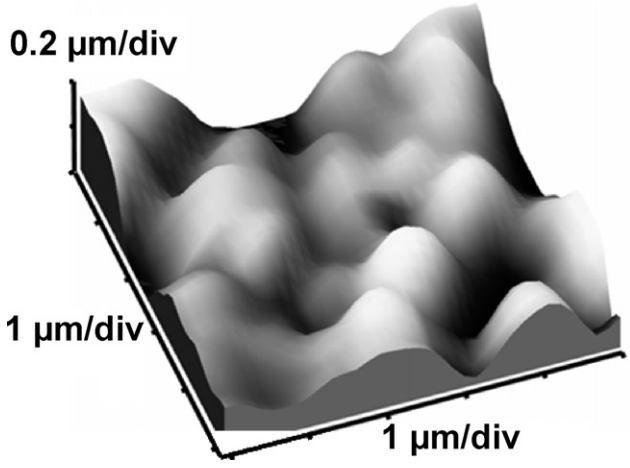

Fig. 2. Tapping mode atomic force microscopy three-dimensional image of the untreated hydroxyapatite sample. No significant topographical changes are found on the irradiated or annealed hydroxyapatite samples in comparison to the untreated samples.