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Electron-induced surface modification of hydroxyapatite-coated implant

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Abstract

Here, the authors report on surface free energy modulation of a hydroxyapatite-coated titanium femoral implant that is performed by a newly-developed method using a low-energy electron irradiation. They observe pronounced increase of hydrophobicity of irradiated samples that occurs in several stages and is characterized by various mechanisms. Bacterial adhesion on electron modified hydroxyapatite samples is studied, by considering different approaches. The authors show that bacterial adherence is selective and depends on the surface free energy components, which were determined from detailed surface free energy analysis. The selective bacterial adhesion, together with the ability to define the surface energy properties, suggests that this newly-developed method opens an avenue for protection of implants from bacterial infections.

Keywords: Hydroxyapatite coating; Surface analysis; Surface energy; Bacterial adhesion; Electron beam; Surface modification

1. Introduction

Development of biomaterials, inherently resistant to bacterial adhesion and growth, is a key challenge in the field of medical implants [1]. Analysis of numerous publications presented in the review papers [2–4] shows that bacterial adhesion is mediated by a large variety of biological processes and critically depends on the bacterial–biomaterial interface properties. The susceptibility of biomaterials to bacterial adhesion is determined by several factors, such as the chemical composition of the surface, fine surface topography, roughness, surface electric charge and wettability state [5]. Among the various physical properties of material substrates influencing bacterial adhesion, wettability and electric charge are likely to be the leading factors [2].

Bacterial adherence to surfaces is not fully understood. In general, it is influenced by many factors, such as growth medium, age of the culture, physiological state, and cell surface [3,4]. In general, hydrophobic bacteria adhere on hydrophobic surfaces, whereas hydrophilic ones prefer hydrophilic surfaces [3,4]. Thus, adjusting substrate wettability, which affects the hydrophobicity, is expected to affect bacterial adherence and can also be a key for understanding bacterial interaction with material surfaces. Such a modification of the surface wettability may open the avenue for protection of implanted scaffolds from bacterial infections.

The basic intrinsic properties of the biomaterials, which define their interaction with various biological cells, can be modified to affect the interaction of the biological cells with the surface [6]. Previously used methods for variation of surface/biological cells interactions included: deposition of self-assembled monolayers, external electric potential application, light-induced changes and electrochemical methods [6]. These methods result in changes of the surface properties and in corresponding variation of the wettability in "on/off" switching mode.

We have developed a new approach to modify material surface wettability by a low-energy electron irradiation [7,8]. This approach is based on trapping of injected and generated electron/hole charges (primary incident electrons and secondary electron/hole pairs) results in modification of the electric potential in the vicinity of the surface leading to a strong variation of the surface energy and related properties (adhesion, adsorption, wettability, *etc*) [7]. The method provides gradual modulation of the wettability in a wide range of contact angles from hydrophilic

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Table 1
Surface energy [35] and density characteristics of used probe liquids at 20 °C

Liquids	Supplier	Purity (%)	Density (kg/m^3)	Surface free energy (mJ/m^2)				
				γ^{LW}	γ^+	γ^-	$\gamma^{AB}=2\sqrt{\gamma^+\gamma^-}$	$\gamma_{lv}=\gamma^{LW}+\gamma^{AB}$
Water		De-ionized	998	21.8	25.5	25.5	51.0	72.8
Diiodomethane	Alfa Aesar	99	3322	50.8	0	0	0	50.8
Ethylene glycol	Aldrich	99	1113	29.0	1.9	47.0	19.0	48.0

(water contact angle $\theta=10°$) to pronounced hydrophobic state ($\theta=100°$) by variation of the incident electron charge. The developed method based on the electron irradiation technology allows high-resolution wettability patterning on irradiated samples [8]. Such a modification has resulted in observation of selective adhesion of biological cells on a hydroxyapatite (HAp) surface [9]. It has been shown that hydrophobic protein (bovine serum albumin) adheres on the hydrophobic part of the wettability patterned HAp sample, while hydrophilic DNA adheres on its hydrophilic part. This method of flexible wettability tuning and patterning has been applied to other solid state materials including metals, which are always coated by native oxide films or other dielectric layers. Our experiments with various calcium phosphates, Ti, crystalline and thin film Al_2O_3, glass, Si, mica, Si_3N_4 and SiO_2 amorphous thin films, and Si-nanocrystals embedded into SiO_2 matrix showed similar behavior of the wettability under a low-energy electron irradiation.

Here we present the experimental data on tunable wettability engineering of a HAp-coated implant by a low-energy electron irradiation. The selective adhesion of three various bacteria to the electron modified HAp-coated surfaces have been observed. We analyzed bacteria and HAp surface properties, which may be associated with mechanism of bacterial affinity to the medical implants with HAp coating.

2. Materials and methods

2.1. Studied materials

In this research we studied commercially available, standard cement-less femoral implants produced from titanium alloy and coated with HAp, using a vacuum plasma spraying technique. The implant was split into plate-like samples (15×10mm).

2.2. Materials surface modification

Surface modification was performed by a low-energy electron irradiation of the preliminary cleaned implant samples using a commercially available electron gun (Kimball Physics Inc., USA) in vacuum 10^{-7}Torr. To locate the electron irradiated region inside the HAp surface layer which is the depth of depleted region (region of band bending), the electron energy was estimated [10] around $E_p \sim 100$eV, which provides the region of the electron excitation at the depth below 20Å. The calculations of the electron penetration performed by the use of the Monte-Carlo method were consisted with analytical solution [10]. The incident charge Q was varied, ranging from 0 to 200 μC/cm^2, to fabricate the required surface wettability state [9].

2.3. Materials surface characterization

X-ray diffraction (XRD) (Scintag, USA) and high-resolution X-ray photoelectron spectroscopy (XPS) (5600 multi-technique system, PHI, USA) were applied to control structure and chemical composition of the HAp-coated samples. Topography and roughness features were inspected by atomic force microscopy (AFM) (Multimode, DI, USA) in a tapping mode in air, using standard Si tip. Additionally, the HAp-coated samples were imaged by conventional scanning electron microscopy (SEM) using a Raith 150 ultra high-resolution e-beam tool (Raith, GmbH Germany). A band-bending variation in the HAp-coated samples, induced by a low-energy electron irradiation, was studied by measurements of current–voltage (I–V) characteristics in the nanometer scale using tunneling AFM (TUNA). The measurements were performed by the use of conductive diamond-coated Si tip. The current was measured at a voltage bias, ranging from −4 to +4V. The TUNA measurements allow determination of the HAp surface potential as a function of incident electron charge.

2.4. Materials wettability

The basic measurements of the wettability were performed by controlling static contact angles of sessile drops of de-ionized water (pH=5.5 and resistivity was more than 17MΩ cm) placed on the sample surface. Additional studies were also implemented by the use of two additional probe liquids, such as Ethylene glycol and Diiodomethane (Table 1). The effect of surface heterogeneity of the studied samples was examined by measuring contact angle

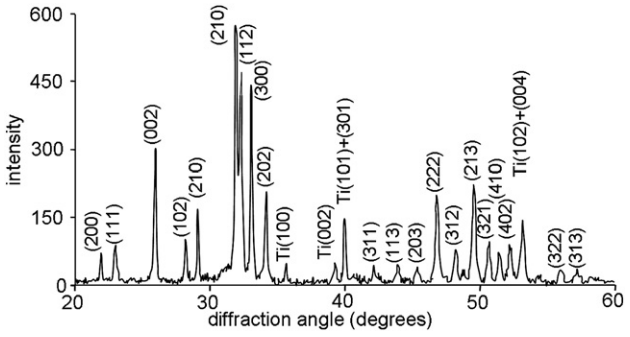

Fig. 1. X-ray diffraction pattern for the hydroxyapatite-coated sample.