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A model of adsorption of albumin on the implant surface titanium and titanium modified carbon coatings (MWCNT-EPD). 2D correlation analysis

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A B S T R A C T

Common materials used as orthopedic implants are titanium and its alloys. To improve its compatibility with the environment of a living organism titanium implant surfaces are covered with bioactive layers of MWCNT. During the insertion into a living organism such material is exposed to direct contact with the patient's blood, which includes proteins – eg. albumin. The adsorption of albumin may constitute one of the early stages of implant surface modification serving cell adhesion. An analysis of this phenomenon in terms of the kinetics of deposition of protein on the surface of the implant confirms its biocompatibility *in vivo*.

The proposed working model of the adsorption of albumin allows for choosing the best of time for the protein to form a stable connection with the surface of the titanium implant. Traditional methods of materials engineering and chemistry allow for the obtaining of information about the presence of a protein on the surface (UV–Vis, the wettability). The application of 2D correlation analysis, in turn, gains insight into the dynamics of the changes associated with the deposition of protein (the formation of a uniform layer, the change in conformation). This analysis has allowed for the selection of an optimal time of protein adsorption to the surface of the implant. Better compatibility with the body of the implant provides its modification by introducing layers that accelerate the material–tissue interactions. Such a composition is a layer of carbon nanotubes (MWCNTs) deposited on titanium by the electrophoretic (EPD) method. Using Raman spectroscopy and analyzing the spectra with the 2D correlation method it is possible to gain insight into the molecular structure of this layer.

Our studies indicate that albumin in contact with the surface of titanium has obtained stable conformation after 30 min (confirmed by: UV–Vis, Raman). Shifts of the CH_2, CH_3 stretching bands position as well as an analysis of the amide I band confirms this conformation. The dynamics of these changes are noticed as correlation peaks observed on 2D maps.

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1. Introduction

Progress in medical diagnosis, treatment, and rehabilitation would not be possible without the continuous discovery and introduction of new or improved materials [1]. Developments in the field of biomaterials has significantly contributed to cardiovascular care, orthopedics and dentistry, etc. Biomedical materials are of natural origin or synthetic, whose main task is the treatment, diagnosis, improvement or replacement (partial or complete) of a patients damaged tissues and organs. For all implanted biomaterials and biodevices, the vital characteristics are their biofunctionality and biocompatibility [2].

For these reasons, the main types of biomaterials currently used are: metals [3,4], natural and synthetic polymers [5–7], and carbon, composites and bioceramic materials [8,9].

Metallic biomaterials are considered for orthopedic, spinal, dental, cardiovascular, neural, and urological applications mainly due to their mechanical properties. A common material used as

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orthopedic implants is titanium, which is regarded as the most biocompatible metal [10]. The host response to biomaterials is largely determined by the surface or interfacial properties of the implanted material [11,12]. After implantation in the body the protein adsorption process occurs, which lasts from a few seconds to 1 h. This is a process of bio-film formation, that is, the deposition of protein on the surface of the implant.

To improve its compatibility with the environment of a living organism a titanium implant's surface is covered with bioactive layers of: ceramic (hydroxyapatite, HA) or carbon (eg. MWCNT) [3,8]. This new generation of materials with carbon nanotubes can be used as sensors and neural electrodes [3,4]. During the insertion into a living organism such material is exposed to direct contact with the patient's blood, which includes proteins – eg. albumin [13]. The adsorption of albumin may constitute to one of the early stages of implant surface modification serving cell adhesion. An analysis of this phenomenon in terms of the kinetics of deposition of protein on the surface of the implant confirms its biocompatibility *in vivo*.

The first objective of this study was to define the surface parameters of the MWCNTs coating formed on the titanium support, that best meets the requirements of biocompatibility. An analysis of this goal was carried out using the methods of Raman microspectroscopy and two-dimensional (2D) correlation analysis. The second task was to verify for such specific layer the parameters relevant in medical applications. We applied traditional methods of materials engineering and chemistry to obtain information about the presence of a protein on the surface (UV–Vis, the wettability). Additionally applied Raman microspectroscopic techniques enabled probing materials and tissues, and also an interface between materials and tissues. The 2D Raman correlation method allows for an analysis of Raman spectra of the system that responds to external perturbation, and finds the relationships between components of the system.

2. Experimental

2.1. Preparation of the sample

The MWCNTs (outside diameter 10–30 nm; inner diameter 5–10 nm, length of 1–2 μm and more than 95% purity) were purchased from Nanostructured & Amorphous Materials, Inc., USA. Carbon nanotubes were oxidized in a mixture of concentrated acids (H_2SO_4 and HNO_3). A sequence of washing with distilled water, centrifuging and finally drying in 40 °C resulted in obtaining highly oxidized, dry CNTs. The presence of carboxylic groups on the surface of the MWCNTs, after oxidation, provides a negative charge on the surface and makes the electrophoretic deposition (EPD) possible.

To obtain the suspension of the resulting oxidized CNTs, they were dispersed in ethanol, acetone and water using a ultrasonic processor (Vibra-Cell, type VCX130, from Sonics & Materials, Inc, USA). The resulting suspension was then used in the electrophoretic deposition EPD method to cover titanium (Ti) plates of 10 × 10 mm size with the MWCNTs layer. The setup for the EPD process consists of a DC power supply (EL561R from Thurlby Thandar Instruments Ltd, UK) multimeter (34405A, Agilent, USA), electrolytic bath and a stainless steel counter electrode. A carbon nanotube deposition was performed for 2, 10, 30 and 60 s, using a voltage of 30 V with titanium plate as a positively charged electrode. Afterwards, the samples were left to dry in room atmosphere conditions.

The plates of pure titanium before incubation were dipped for 10 min in distilled water with soap and cleaned using an ultrasonic washer. For the next 10 min the plates were again subjected to an ultrasonic treatment, but this time they were immersed in acetone. After drying, the plates were incubated in 1% solution of chicken egg white albumin, the first series, and human serum albumin, the second series, for 15, 30, 45, 60, 75 and 90 min.

Additionally, the Ti plates with MWCNTs coating (30 s deposition time in the EPD process) were incubated in solution of chicken egg white albumin and human serum albumin, for 90 min and 30 min, respectively. Both solutions were of 1% albumin concentration.

The albumin from chicken egg white and human serum albumin were purchased from Sigma–Aldrich (Poland).

2.2. Raman microspectroscopy

Raman spectroscopy measurements were performed using a Renishaw inVia Raman spectrometer working in confocal mode, with 100× magnification objectives (NA = 0.90). Samples were excited with the 514.5 nm laser line.

The measurement configuration was as follows: exposure time: 10 s, accumulations: 9, laser power ~1 mW to avoid heating of the sample.

Factory supplied software was used to analyze the spectra, calculate the position, intensity, and area of the characteristic peaks (Renishaw, WiRE v. 2.0 and 3.4).

2.3. 2D Raman correlation

The generalized 2D correlation analysis based on the Noda's method [14–16] was performed using Raman spectra as an input data for generating the correlation maps. 2Dshige, v.1.3 software was employed [17].

2.4. UV–Vis spectroscopy and contact angle measurements

UV–Vis measurements were performed on a Cecil CE 2502, 2000 Series, spectrometer at a wavelength of 280 and 290 nm. Two series of measurements were performed for the Ti plates incubated in chicken egg white albumin, as well as in the human serum albumin. The first series of analysis consisted of the absorbance measurements of the solution remaining in the cuvette after the incubation of the plates. The second series, to verify results, involved measuring the absorbance of albumin which were desorbed from the titanium plates.

The contact angle measurements were performed on a SAM10Mk1 (KRÜSS GmbH, Germany) goniometer using deionized water. The average value of the contact angle has been determined based on a minimum of 5 results.

3. Results and discussion

3.1. The structure of the MWCNTs coatings

3.1.1. Raman microspectroscopy

Multi-walled carbon nanotubes were used as a modifying phase for the surface of the biocompatible titanium plates. Photomicrographs of the obtained coatings surface formed in 2, 10, 30 and 60 s deposition time in the EPD process is shown in Fig. 1. The surface morphology of the samples is very much dependent on deposition time. Carbon nanotubes coating is uneven, features like rafts and valleys are apparent. The thickness and shape of the coating created in the 30 s deposition time seems to have the most interesting topography, for the contact body fluids including blood.

The Raman measurements taken at the top surface of the MWCNTs coating obtained in different deposition time is presented in Fig. 2 (Table 1). The position of the G band at ca.1580 cm^{-1} is

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