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Short communication

Primary role of electron work function for evaluation of nanostructured titania implant surface against bacterial infection

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ABSTRACT

The electron work function as an essential descriptor for the evaluation of metal implant surfaces against bacterial infection is identified for the first time. Its validity is demonstrated on *Staphylococcus aureus* adhesion to nanostructured titania surfaces. The established correlation: work function–bacteria adhesion is of general importance since it can be used for direct evaluation of any electrically conductive implant surfaces.

1. Introduction

Titanium and Ti-based alloys are widely used as biomaterials due to their unique properties such as high strength, low density and excellent corrosion resistance [1]. Despite good biocompatibility of Ti-based materials, bacteria may colonize the implant during the initial 'race for the surface' [2]. Therefore, an appropriate selection and surface engineering of the implant material are the key factors for long-term success. Such measures are necessary to counteract presurgical contamination, which may lead to infection, both immediately or within the first 3 months of implantation [3]. The risk of biomaterial centred infection (BCI) is the main drawback limiting their use [4,5]. To prevent bacteria colonization of the metal implants surface, several approaches which require precise engineering of their surface architecture and properties can be considered e.g. changing surface chemistry and functional groups (e.g. anti-adherent polymers, copolymers, proteins) and/or introducing topographical features on the surface (micro– and nanotubes, pores) [2,6]. All of the mentioned practises aim in favouring osseointegration over hazardous bacteria attack.

One of the state–of–the–art solutions is the fabrication of a nanoporous anodic titanium oxide (ATO). An ATO layer formed on a Ti substrate has several advantages like nontoxicity, biocompatibility and osseointegration. The nanoporous structure of ATO positively affects adhesion, spreading, growth, and the differentiation of osteoblast cells [7,8]. Additionally, the pores can be used as potential drug reservoirs for a controlled delivery system. Implant surface tailoring introduces not only topographical changes but also surface chemistry, thus, it is a cornerstone for enhanced implant biocompatibility. Such changes are a subject of a vast number of scientific papers devoted to microscopic (SEM, TEM, CM, AFM) and spectroscopic (LDI-MS, XPS, DRIFT, μRS) implant surface characterization. However, the most sensitive technique for monitoring the changes in surface electronic properties, work function measurements by means of Kelvin probe, have never been applied in this context. The work function is defined as the smallest amount of energy required to remove an electron from the surface (Fermi level) to a point in a field-free zone just outside the solid (vacuum level) [9]. It is commonly used as a fundamental parameter describing the ability of surfaces to adsorb molecules and stimulate catalytic reactions where redox processes (electron transfer) take place [10,11].

Microbial adhesion to implant surfaces is primarily mediated by non–specific interaction forces which include Lifshitz–Van der Waals forces and electrostatic forces, which both operate over a long range, as well as hydrophobic and acid-base interactions that act over a shorter range [12]. After approaching the implant surface, microorganisms are attracted or repelled by the biomaterial surface, depending on the resultant interaction forces. In orthopaedic devices BCI is caused mainly by 3

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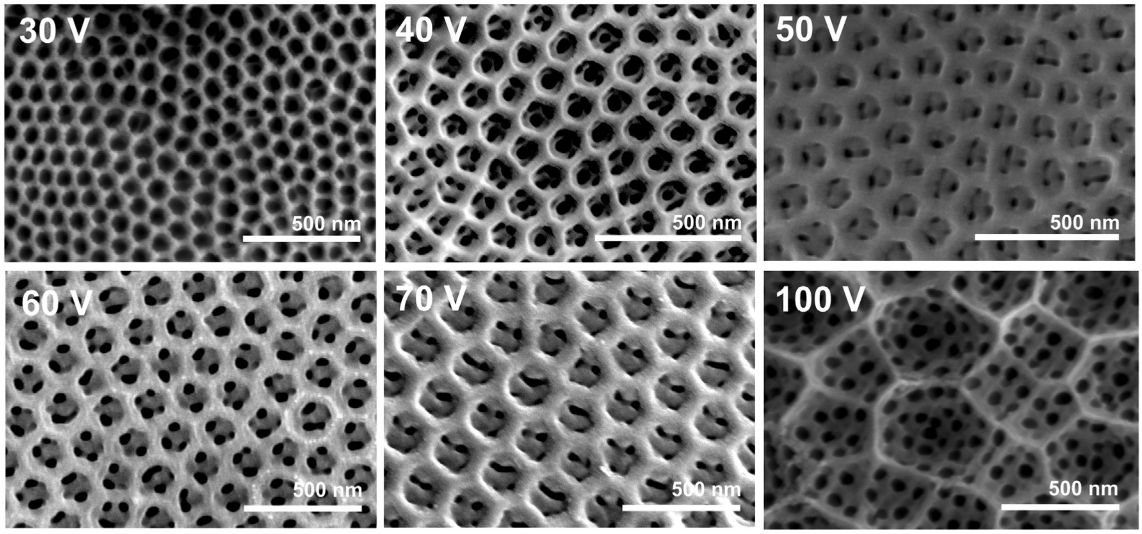

Fig. 1. SEM images of ATO layers of various morphologies obtained at different potentials 30–100 V.

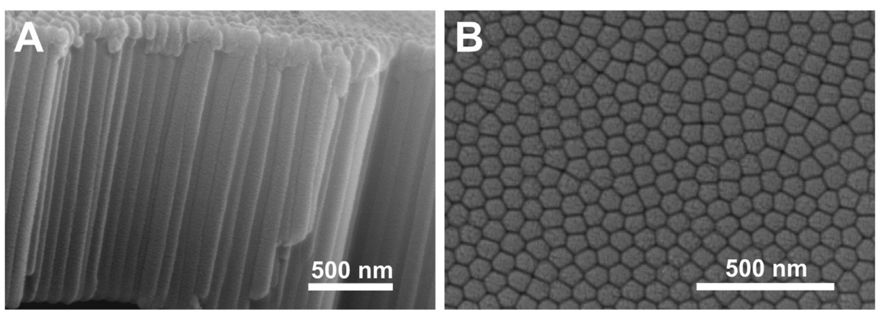

Fig. 2. Typical morphology of nanoporous anodic TiO_2: (A) cross-sectional and (B) bottom views of the sample obtained in the ethylene glycol based solution at 30 V, 20 °C and stirring speed of 200 rpm.

strains: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* [13]. *S. aureus* is considered as a model strain in studies of bacterial adhesion as well as biofilm formation on biomaterial surfaces, and thus its features are well described in microbiological literature [14]. The cell envelope of *S. aureus* has been studied most extensively because of interest in its role in the interaction with the environment in the course of infection [15]. *S. aureus*, as a Gram–positive bacterium, is surrounded by layers of peptidoglycan and long anionic polymers called teichoic acids (TA). There are two major types of TA: wall teichoic acids (WTA) which are coupled with peptidoglycan and lipoteichoic acids (LTA), which are anchored to the cell membrane. These polymers can account for over 60% of the mass of Gram–positive cell wall, making them major contributors to envelope structure, function and charge [16]. Moreover, the *S. aureus* colonization of abiotic surfaces depends on the partial charges accumulated on TA functional groups [17].

In this paper, we propose to apply the work function as a direct parameter for evaluation of surfaces to adsorb bacteria. This conjecture is based on the fact, that during the adsorption process the electrostatic interaction between the material surface and the bacterial cell wall (with a negative net charge) is of vital importance. The applied experimental conditions are relevant for the initial period of implantation, where the adsorbing biological moieties such as proteins are absent. At this stage of the 'race for the surface' phenomenon, there is the highest probability of bacterial infection. To our knowledge, the electron work function is applied for the first time for the direct evaluation of implant surfaces against bacteria attachment. For this purpose, we chose: *S. aureus* as the most common strain in orthopaedic infections and titania surfaces as the most popular interphase of metal implants.

2. Materials and methods

2.1. Samples preparation

Ti foil (99.5% purity, thickness 0.25 mm) supplied by Sigma-Aldrich was used as a substrate for the preparation of nanoporous TiO_2 samples. Titanium specimens (1×1 cm) were cleaned, then polished electrochemically and chemically as described elsewhere [18,19]. Nanoporous anodic titanium oxide layers were prepared by a three-step anodization

Table 1
Surface geometric characteristics of tested samples.

Anodization potential	Pore diameter nm	Subpore diameter nm	Wall thickness nm	Porosity %
30 V	69 ± 15.3	–	29 ± 4	35 ± 4
40 V	100 ± 24.1	46 ± 10	56 ± 6	32 ± 5
50 V	121 ± 20.3	61 ± 6	90 ± 8	29 ± 4
60 V	146 ± 30.9	63 ± 6	84 ± 9	28 ± 6
70 V	157 ± 29.1	67 ± 5	58 ± 10	26 ± 7
80 V	171 ± 38.3	70 ± 6	36 ± 5	20 ± 5
100 V	350 ± 40.3	79 ± 5	30 ± 4	18 ± 3