BACTERIA ADHESION TO GRAPHENIC SURFACES

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Introduction

Graphenic materials, due to their specific properties such as excellent electrical conductivity, inherent mechanical strength, thermal conductivity, affinity for heteroatom insertion and biocompatibility are investigated as a materials for many applications including biosensors, drug delivery systems and implants surface engineering, to mentioned a few [1]. The evaluation of the interaction forces between graphene and bacteria, as well as identification of key parameters ruling the adhesion process are of particular importance [2]. Microbial adhesion to surfaces is primarily mediated by non–specific interaction forces. They include van der Waals and electrostatic potentials, which both operate over a long range, as well as hydrophobic and acid-base interactions that act over a shorter range. The mechanism of bacteria attachment to surfaces can be analysed in terms of DLVO (Derjaguin–Landau–Verwey–Overbeek) theory [3]. In this work, we propose the work function measurements by Kelvin probe as a straightforward method for direct evaluation of graphenic surfaces for bacteria colonization. The changes in work function were induced by controlled functionalization of the graphenic surface by oxygen plasma (introduction of surface oxygen groups).

Materials and Methods

The conductive graphene sheets were purchased from Graphene Laboratories, Calverton NY, USA. The oxygen plasma treatment of graphenic samples was carried out using a Diener electronic Femto plasma system (Diener Electronic GmbH, Nagold, Germany). The partial pressure of oxygen, exposure time and the generator power were adjusted to obtain different levels of modification of the electrodonor properties of graphenic surfaces. The contact potential difference (V_{CPD}) measurements were carried out by the Kelvin method with KP6500 (McAllister Technical Services). The modified surfaces were characterized in terms of carbon structure (μRaman spectroscopy), surface morphology (scanning electron microscope), wettability (water contact angle) and surface oxygen concentration (X-ray photoelectron spectroscopy). The changes in bacteria adhesion (*Staphylococcus aureus* 24167 DSM, Gram-positive bacteria) on the graphenic surfaces after oxygen plasma treatment were investigated using a fluorescent microscope (IX51 Olympus). Microbiological tests were performed for three independent series according to the procedure described elsewhere [4]. After observations, 15 randomized fluorescence microscopic images were taken and the area occupied by the bacteria was evaluated using ImageJ 1.50 V software [5].

Results and Discussion

The graphenic surface was modified by oxygen plasma treatment with various parameters (power: 2-60 W, oxygen pressure: 0.14-0.5 mbar, time: 2 s-20 min). The measured Raman spectra of the investigated graphenic materials are typical with the characteristic bands at: ~1580 cm^{-1} (G band) and ~2725 cm^{-1} (2D band). For the samples exposed to plasma for 5 and 20 min, the D band at ~1350 cm^{-1} appears. The XPS results show a substantial increase in oxygen surface concentration from 0.75 at.% (unmodified graphene) up to 6.6 at.% for the longest time of exposure to plasma (t = 20 min, P = 60 W, p = 0.5 mbar). Generation of surface oxygen groups leads to dramatic increase in the work function (from 4.4 eV to 6.0 eV) and decrease in the water contact angle (from 93.8° to 7.0°). In order to check how the surface modification influences the microbiological properties of the graphenic material, the investigated surfaces were evaluated in terms of bacterial colonization. The quantification of the bacteria adhesion to the graphenic surfaces was performed using the surface area occupied by the *S. aureus* after the incubation time of 1 hour (relevant for the initial stage of biomaterial-centered infection after implantation). The bacteria coverage systematically increased with the plasma treatment from 3.2% (untreated graphenic surface) to 9.2% (60 W, 0.5 mbar, 20 min). The trend of bacteria adhesion for the investigated graphenic surfaces follows the tendency observed for the work function modifications (FIG. 1). The obtained results revealed that colonization of graphenic surfaces is strongly governed by the surface oxygen concentration, wettability, and electronic properties (work function).

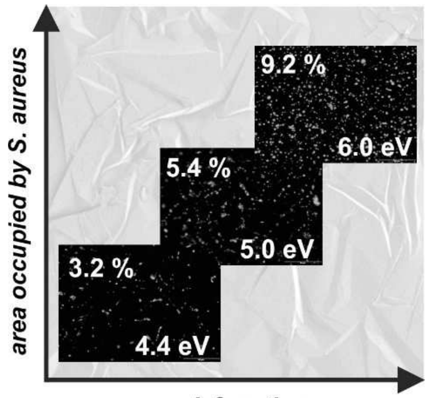

FIG. 1. The general trend observed for *S. aureus* adhesion to graphenic surfaces. At the background the morphology of the graphenic material is shown.

Conclusions

The strong correlation between *S. aureus* adhesion to the graphenic surfaces and electron work function of the prepared samples was discovered. Based on the obtained results the electron work function was proposed as a suitable parameter for the evaluation of graphenic surfaces against bacteria colonization. Introduction of the surface oxygen groups leads to dramatic changes in wettability and work function, so their surface coverage should be controlled, while designing the carbon-based biomaterials.

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