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Short communication

Tritrichomonas foetus adhere to superhydrophilic vertically aligned multi-walled carbon nanotube surface

Susane Moreira Machado [a], Anderson Oliveira Lobo [b,c,*], Ariel Bueno Loureiro Sapucahy [a], Fernanda Roberta Marciano [b,c], Evaldo Jose Corat [d], Newton Soares da Silva [a]

[a] Laboratorio de Biologia Celular e Tecidual, Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, Av. Shishima Hifumi 2911, 12244-000, Sao Jose dos Campos, SP, Brazil
[b] Laboratorio de Nanotecnologia Biomedica (NanoBio), Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraiba, Av. Shishima Hifumi 2911, Sao Jose dos Campos, 12244-000, SP, Brazil
[c] Laboratorio de Espectroscopia Vibracional Biomedica, Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraiba, Avenida Shishima Hifumi, 2911, CEP 12244-000, São José dos Campos, SP, Brazil
[d] Instituto Nacional de Pesquisas Espaciais (INPE), Laboratorio Associado de Sensores e Materiais (LAS), Av. dos Astronautas 1758, Sao Jose dos Campos, 12227-010 SP, Brazil

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ABSTRACT

For the first time, we show that *Tritrichomonas foetus* can adhere on superhydrophilic vertically aligned carbon nanotubes (VACNT) films. Scanning electron microscopy shows an unusual adhesion with a higher membrane filopodium projection in all directions, directly attached to superhydrophilic VACNT tips.

1. Introduction

Tritrichomonas foetus (*T. foetus*) is the causative agent of cattle trichomonosis, one of the most prevalent sexually transmissive infections in cattle. In cows, the infection varies from mild vaginitis or cervicitis, to endometritis, abortion and infertility. Significant losses may occur because of infertility and abortion [1,2]. Neither the cellular processes by which *T. foetus* colonizes mucosal surfaces nor the mechanisms for tissue damage are well defined. Thus, there is a need for a deeper understanding of the pathogenic effects of this parasite to better describe the host–parasite correlation. Many simple cell lines allowed the study of the trichomonad–host interaction. Examples of these cell lines are chicken embryo explants [3], HeLa and McCoy cells [4,5], Vero cells [3,5], RK-13 cells [6], CHO cells [2], WISH cells [7], MDCK cells [8–10], baboon testes, and others [5,11,12].

Carbon nanotube (CNT) can absorb bacteria [13–16] and show strong antimicrobial activity toward *Escherichia coli* [17,18]. It has been recognized that hydrophilic surfaces are favorable to the adhesion, spreading and growth of various cell types [19–21]. However, some studies have shown that raw vertically aligned CNT (VACNT) are superhydrophobic [22–24], which may be a limit for their application as nanostructures for cellular and bacterial attachment [25,26]. Lobo et al. [27] showed that a simple functionalization using oxygen plasma was effective to convert VACNT to superhydrophilic.

For the first time, we have shown the *T. foetus* adhesion on superhydrophylic VACNT surfaces. Scanning electron microscopy (SEM) images revealed an unusual sticking to superhydrophilic VACNT films. It seems possible to apply superhydrophilic VACNT films to understand protozoan spreading mechanisms and, the specific recognition of adhesion proteins. This is particularly applicable because of feasibility of VACNT functionalization.

2. Materials and methods

VACNT films produced using a microwave plasma chamber at 2.45 GHz [28,29] were the samples used. VACNT grew on Ti substrates (10 mm × 10 mm × 1 mm) covered by 10 nm Ni layers deposited by e-beam evaporation. The Ni layers were pre-treated to promote nanocluster formation, which served as the catalyst for VACNT growth. The 5 min pretreatment in N_2/H_2 (10/90 sccm) plasma, at a substrate temperature of nearly 760 °C, promoted nano-catalyst formation. VACNT growth started by introducing CH_4 (14 sccm) to the gas mixture, with a substrate temperature of 800 °C. It continued for 2 min. The reactor pressure was 30 Torr during the whole process. An oxygen plasma functionalized VACNT tips by incorporating oxygen-containing groups. The pulsed direct current plasma functioned with

* Corresponding author at: Universidade do Vale do Paraiba (UNIVAP), Instituto de Pesquisa e Desenvolvimento (IP&D), Laboratorio de Nanotecnologia Biomedica (NanoBio), Avenida Shishima Hifumi, 2911, Sao Jose dos Campos, CEP: 12.244-000, SP, Brazil. Tel.: +55 12 3947 1166; fax: +55 12 3947 1166.
E-mail address: loboao@yahoo.com (A.O. Lobo).

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an oxygen flow rate of 1 sccm, a pressure of 80 mTorr, −700 V and a frequency of 20 kHz [30]. The polar groups' attachment on superhydrophilic VACNT tips is better described elsewhere [30].

The contact angle (θ) of the samples was measured by using the sessile drop method with a Kruss EasyDrop contact angle instrument (EasyDrop DSA 100). Two different test liquids (distilled water and diiodomethane) were used for surface energy calculations, according to Owens method [31]. The liquid was dropped automatically by a computer-controlled system. All measurements were carried out at room temperature.

K strains of *T. foetus* isolated from the urogenital tract of a bull were maintained in TYM Diamond's medium, supplemented with Fetal Bovine Serum (10%, Gibco/BRL). A suspension of 10^6 *T. foetus* K cultivated during 48 h, diluted in 1 ml phosphate buffer saline (PBS), with pH adjusted to 7.2. Incubation occurred within a CO_2 (5%) atmosphere at 37 °C.

The superhydrophilic VACNT samples sterilized for 24 h under UV irradiation were placed in individual wells of 24-well culture plates. The suspension containing *T. foetus* was inserted in each well. Incubation took place under a CO_2 (5%) atmosphere, at 37 °C, during 1 h. Tests were performed in triplicate. After incubation period, the *T. foetus* attached to the superhydrophilic VACNT films were fixed with a 3% glutaraldehyde/0.1 M sodium cacodylate buffer for 1 h and dehydrated in a graded ethanol solution series (30%, 50%, 70%, 95%, 100%), for 10 min each. The drying stage used a 1:1 solution of ethanol with hexamethyldisilazane (HMDS) and the samples were dried with pure HMDS at room temperature. After deposition of a thin gold layer, the specimens were examined with a SEM Zeiss EVOMA10.

In order to investigate the parasite adhesion mechanism, work of adhesion for the parasite to attach the coatings was calculated using thermodynamic approach [32]:

$$\Delta F_{Adh} = \gamma_{SP} - \gamma_{SL} - \gamma_{PL} \quad (1)$$

where ΔF_{Adh} is the interfacial free energy of adhesion, γ_{SP} the solid-parasite interfacial free energy, γ_{SL} the solid–liquid interfacial free energy and γ_{PL} is the parasite-liquid interfacial free energy. They can be calculated by using contact angle data and van Oss acid–base approach [32–34].

The following equation is used to determine the interfacial energy of parasite adhesion to a solid surface [32–34]:

$$\Delta F_{Adh} = 2\left(\begin{array}{l}\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}} \\ + \sqrt{\gamma_P^{LW}\gamma_L^{LW}} + \sqrt{\gamma_P^{+}\gamma_L^{-}} + \sqrt{\gamma_P^{-}\gamma_L^{+}} \\ -\sqrt{\gamma_S^{LW}\gamma_P^{LW}} - \sqrt{\gamma_S^{+}\gamma_P^{-}} - \sqrt{\gamma_S^{-}\gamma_P^{+}} - \gamma_L\end{array}\right) \quad (2)$$

According to thermodynamic theory, if ΔF_{Adh} is negative adhesion is thermodynamically favorable. While if ΔF_{Adh} is positive, adhesion is thermodynamically unfavorable.

3. Results and discussions

Fig. 1a and b shows the SEM images of the patterned surface of roughly 500 μm × 500 μm × 10 μm VACNT grown on Ti surfaces. The pattern produced by conventional lithography has irregular borders (Fig. 1a) and the lateral view (Fig. 1b) show that the VACNT clusters are aligned with homogeneous thickness.

Fig. 2 shows the SEM images to evaluate the *T. foetus* adhesion on the superhydrophilic VACNT films, after an incubation period of 1-hour. In Fig. 2a, *T. foetus* adhered to superhydrophilic VACNT tips. White arrows detail this adhesion. Fig. 2a shows the parasite keeps its pear and shape. Notice that the higher number of fillopodia presented by *T. foetus* (arrows). In Fig. 2b, it is possible to see the adhesion region is in the area opposite to the recurrent flagellum (localized by left arrow). All flagella are free with reduced microvillus height. Many fillopodia connect the parasite to the superhydrophilic VACNT films (arrows). Fig. 2c shows *T. foetus* adhered in all regions of the superhydrophilic VACNT films (arrows), which change its original morphology to form finger-like structures, observed as digitopodia (arrows). Digitopodia are seen as membrane specializations in the adhesion region of all *T. foetus* structures (arrows). *T. foetus* preserved its tear-drop shape with all of its flagella externalized and the recurrent flagellum placed on the topside (Fig. 2b–d). The posterior tip firmly adhered to the superhydrophilic VACNT films (Fig. 2b and c) since successive washes (because of methodology for SEM analysis) did not detach them from superhydrophilic VACNT films. Fig. 2d distinguishes the very healthy *T foetus* behavior as seen by the active formation of membrane projections all over the surface.

The surface energy values for as-grown VACNT were 50.5 mJ m^{-2}, with 48.7 and 1.8 mJ m^{-2} for dispersive and polar parts. Remarkably, there is a huge increase on polar part of surface energy after the oxygen plasma treatment and this is the main reason for describing it as polar-VACNT. These measurements show clearly the polar groups grafting by the O_2 plasma efficiently changes polar part of surface energy. This change on polar part of surface energy is responsible for the change from a superhydrophobic to a superhydrophilic character. From a physicochemical point of view, the adhesion of parasite to a surface is determined by the interplay of electrostatic and hydrophobic/hydrophilic interactions [35]. The interfacial free energy determines the wetting characteristics and hence the wall shear stress generated when the liquid comes into contact with the surface [31]. The average value of work of adhesion (ΔF_{Adh}) for superhydrophobic samples (VANCT) was −7.6 mJ m^{-2}. This value reduces to −11.8 mJ m^{-2} when functionalized VACNT were used.

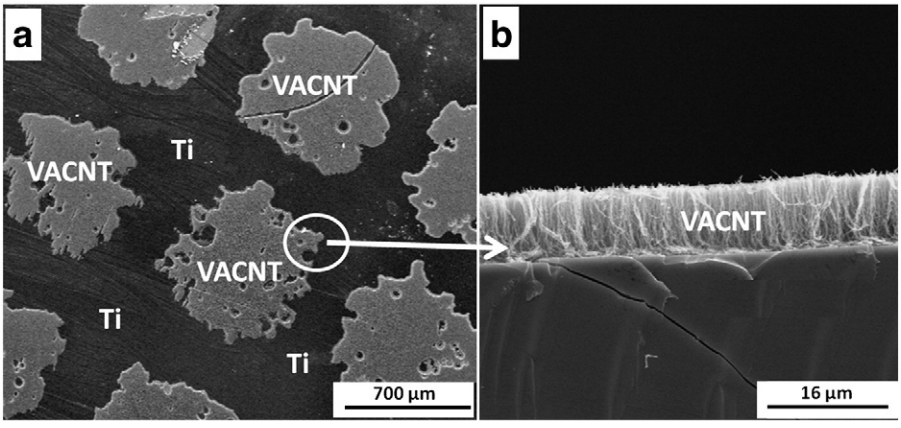

Fig. 1. SEM images of (a) conventional lithography pattern used to create a pattern of 500 μm × 500 μm × 10 μm for allowing a right comparison between VACNT and Ti surfaces. (b) Thickness details of superhydrophilic VACNT films.