Materials Science and Engineering C 80 (2017) 117–121

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Bactericidal performance of nanostructured surfaces by fluorocarbon plasma

E. Vassallo [a,*], M. Pedroni [a], T. Silvetti [b], S. Morandi [b], S. Toffolatti [d], G. Angella [c], M. Brasca [b]

[a] CNR, Institute of Plasma Physics, Via R. Cozzi 53, 20125 Milan, Italy
[b] CNR, Institute of Sciences of Food Production, Via Celoria 2, 20133 Milan, Italy
[c] CNR, Institute of Condensed Matter Chemistry and Technologies for Energy, Via R. Cozzi 53, 20125 Milan, Italy
[d] University of Milan, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Italy

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 8 May 2017
Accepted 16 May 2017
Available online 19 May 2017

Keywords:
Nanopattern
Bactericidal
Antibiofouling
Escherichia coli
Staphylococcus aureus
Bacillus cereus

ABSTRACT

This study presents the characterization and antibacterial activity of nanostructured Si by plasma treatment method using a tetrafluoromethane (CF_4) and hydrogen (H_2) mixture. Nanostructured-Si is a synthetic nanomaterial that contains high aspect ratio nanoprotrusions on its surface, produced through a reactive-ion etching process. We have shown that the nanoprotrusions on the surfaces produce a mechanical bactericidal effect. Nanostructured-Si exhibited notable activity against three different microorganisms: Gram-negative (*Escherichia coli*), Gram-positive (*Staphylococcus aureus*) and spore-forming bacteria (*Bacillus cereus*) producing a > 5 $\log_{10}$ reduction after 24 h of incubation.
Scanning electron microscopy was used to analysis the structure and morphology character of different surfaces evidencing the physical bactericidal activity of the Nanostructured-Si. These results provide excellent prospects for the development of a new generation of antibacterial surfaces.

1. Introduction

Microbial infection remains one of the most serious complications in several areas, particularly in medical devices, drugs, hygienic applications, water purification systems, textiles, food packaging and storage, and filters used in air-conditioning systems [1]. Both antibiotic-resistant Gram-negative and Gram-positive bacteria are reported to be important causes of bacterial infections [2–3]. Recent years have seen increased development of nanoparticle designs as treatments for various diseases and infections [1]. The antibacterial activity of inorganic materials is of significant interest due to the need for infection control and rising antibiotic resistance. Bacterial adhesion depends on the solid surface properties (roughness, solid surface, chemical structure, hydrophobicity, surface charge, etc.). Ivanova et al. [4] have shown that a suitably nanostructured surface can kill the bacteria based on texture alone. They had previously observed that cicada wings possessed a strong bactericidal activity against *Pseudomonas aeruginosa* (human pathogen) and showed that surface of the wings was covered by an array of regularly spaced nanopillar structures. Studies have demonstrated that these structures can be replicated in the laboratory, with engineering techniques [5]. Based upon this understanding, Ivanova et al. have reproduced a similar texturing on different materials demonstrating that the bactericidal nature was independent of the biochemical functionality of the wing. They showed that bactericidal nature of the wing was due to the mechanical rapture of bacterial cells. On the basis of this, materials with similar surface topologies have the same bactericidal effect. In literature [6], and also in Ivanova et al. research, the Si texturization has been studied mainly in sulfur hexafluoride (SF_6) and oxygen (O_2) mixture. Although this mixture is very used for processes in microelectronics and for biomedical applications, actually it is virtually impossible to be used because of the sulfur that stinks the treated material. Therefore, in addition to the texturing process by plasma, a cleaning process is necessary, resulting in additional costs and longer time of industrial production. To overcome this problem, in this paper, we report a method which utilizes tetrafluoromethane (CF_4) gas diluted with hydrogen (H_2). The process is realized in a low-density capacitively-coupled plasma RIE reactor [7]. The antibacterial activity of the nanostructured-Si against Gram-negative (*Escherichia coli* ATCC 8739), Gram-positive (*Staphylococcus aureus* ATCC 19095) and spore-forming

* Corresponding author.
E-mail address: vassallo@ifp.cnr.it (E. Vassallo).

http://dx.doi.org/10.1016/j.msec.2017.05.111
0928-4931/

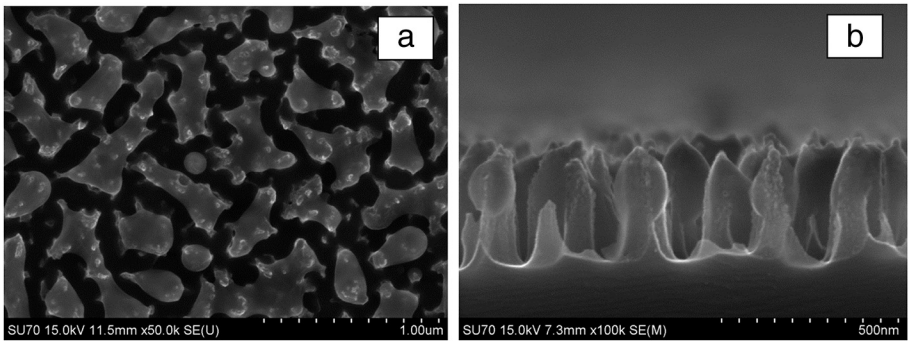

Fig. 1. SEM pictures of Si wafers processed at 200 W of RF Power: (a) top view and (b) cross-sectional view SE imaging with upper detector.

bacteria (*Bacillus cereus* ATCC 14579) is also investigated. This study aims to evaluate the antibacterial property of silicon nano-structures in the formulation of new types of bactericidal materials.

2. Experimental

2.1. The plasma reactor

An RF plasma system [8] has been used to produce a physical structuring of Silicon (type P, dopant B, ⟨100⟩, 0.01–0.02 Ohm-cm, 1×1 cm^2, thickness = 400 μm). The experimental apparatus consists of a parallel-plate, capacitive-coupled system, consisting of a cylindrical stainless steel vacuum chamber with an asymmetric electrode configuration. A powered electrode (3-in diameter) is connected to an RF (13.56 MHz) power supply, coupled with an automatic impedance matching unit, while the other electrode (3-in diameter), consisting of stainless steel, is grounded. Si substrates are placed on the powered electrode at 6 cm away from the ground electrode. The substrate temperature is monitored by a thermocouple fixed directly on the substrate. Before the process, the substrates are cleaned by chemical etching solutions (alcohol followed by rinse in deionized water) to remove surface contaminants. The Atomic Force Microscopy technique has been used to check the surface roughness of substrates after cleaning. The RMS roughness was in accordance to manufacturer's data (≤1 nm). The process chamber is pumped to a base pressure below 1×10^{-4} Pa and high-purity reactive gases (CF_4 and H_2) are introduced into the vacuum chamber through a mass flow controller in order to establish the desired working pressure, which is fixed at 9 Pa. The plasma process was performed for 30 min. A power density of 1 W/cm^2 was applied to RF electrode and H_2/CF_4 ratio equal to 0.1 was set in plasma mixture.

2.2. Characterization of the nanostructured surfaces

Nanostructured surfaces in CF_4/H_2 plasma were examined by Scanning Electron Microscope (SEM) analysis through a high resolution SEM Hitachi SU70 with Schottky electron source and secondary electron

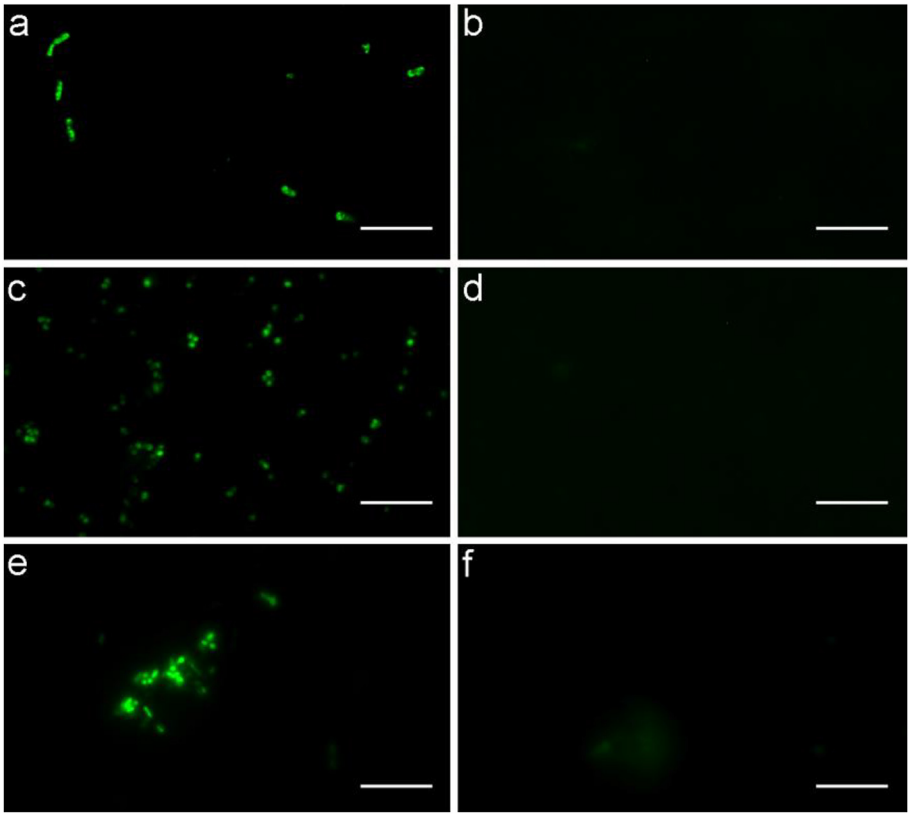

Fig. 2. Video-confocal micrographs showing in green alive *Bacillus cereus* (a, b), *Staphylococcus aureus* (c, d) and *Escherichia coli* (e, f) cells before (a, c, e) and after incubation for 24 h (b, d, f) on nanostructured-Si surfaces. Scale bar: 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)