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Antibacterial properties obtained by low-energy silver implantation in stainless steel surfaces

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ABSTRACT

Biofilm formations on contact surfaces is one of the main causes in several health and food industries due the high incidence of pathogenic contaminations. An approach to reduce problems is to modify the surface properties with antibacterial or antibiofilm agents. The surface modification before biofilm formation reveals to be an effective technological alternative to prevent or eradicate bacterial adhesion in biomedical devices and surgical instruments, as well as food contaminations from process equipments. In this paper we evaluated the antibacterial properties of silver atoms incorporated on stainless steel surfaces by ion implantation at low energy (4 keV) using a reactive low voltage ion plating-type equipment. The simulations by the Monte Carlo method contributed to establish the minimum self-bias voltage used in the implantation process and to provide an ideal distribution estimate of the silver dose in-depth. The surface modifications in all samples were analyzed by RBS, GD-OES and XPS. The microbiological assays were conducted using *Escherichia coli* and *Staphylococcus aureus*, and the results demonstrated an initial reduction on bacterial adhesion, and delay of the biofilm formation. The results help clarify the physicochemical conditions required to obtain a surface resistant to bacteria colonization.

1. Introduction

Bacterial adhesion is the initial step in the colonization and biofilm formation [1]. Several bacteria adhere to surfaces in aqueous environments and form the extracellular polymeric substances (EPS) giving a biofilm structure (i.e., persistent and endemic populations) that provides resistance to antiseptics, antibiotics, and some adverse physical situations [2]. Although the main treatments used for clinical infections are still the antibiotics, their widespread abuse has given rise to a breed of super bacteria that are resistant which is becoming more severe nowadays [3]. The biofilm can be considered as beneficial or detrimental depending on the case, positively contributes in bioprocesses and environmental treatments, while on the other hand, they are detrimental to both human health and several industries (e.g., nosocomial infections and slime formation on industrial devices [4–6]. The complications related to biofilm are considered a risk to public health because of their role in certain infectious diseases and importance in a variety of device-related infections, as well as significant financial losses for industries globally [2,7–9].

The best strategy to attack the biofilm is to prevent its formation [10, 11]. The surface modification/functionalization of materials is the first step to such strategy [12], and the investigation of the biofilm formation mechanism is a crucial factor for the development of materials with antibacterial or antibiofilm properties [1–2]. Consequently, the surface modification of materials aims at controlling the biofilm impact, not only in engineering issues but also in health aspects, such as the food industry and biomedicine [8–10].

Surface modifications should provide a variety of biological responses to the material surface. The surface is the interface where these materials meet and interact with the biological environment (i.e., bone, soft tissue, microorganisms and blood), thus the foremost challenge is improving the function and lifespan in the surface treatment to suit a specific application [12–14]. Alternative technologies including molecular microbiology and engineering surfaces have been proposed for control biofilms. Among these technologies stands out the used specific enzymes that prevent adhesion and the structural integrity of biofilms [15], the use of bacteriophages reduce bacterial proliferation [16], oligodynamic action of antimicrobial agents as quaternary ammonium salts, synthetic antibiotics, and antimicrobial peptides (AMPs), and the modification of surfaces [17–21].

The ion implantation is an important technique for being the most accurate and sophisticated among those mentioned, enabling optimum

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control of deposition and leakage of implanted element [22–25]. This process of physical surface modification injects accelerated high-energy ions into the surface of a material to modify its physicochemical and biological properties [14]. The ion kinetic energies that interact with the target material are between 4 and 5 orders of magnitude larger than the binding energy of the solid material [22,23]. Due the burgeoning threat of multidrug resistance and the dearth of new antibiotics, the implantation of antimicrobial metals to modify the surface of biomaterials [26] and surgical instruments has renaissance in the last decades [18]. The ions most used for this type doped biomaterials are Ag^+, Zn^+ and Cu^+ [18,26–28], and the energy can range from 30 to 200 keV, with some devices allow a greater range comprising values between 2 and 1000 keV [27–29]. The applied doses vary from 1×10^{16} to 5×10^{17} atoms*cm^{-2} and offer an effective increased of bacterial *anti*-adhesive properties [9,13,25,27–29], according to the crystalline structure of substrate.

Although there are many studies in order to modify the surface solid by ion implantation by the ion/target interaction in high energy (≥500 keV) and intermediate energy (30 to 500 keV), there not studies with the use at low-energy regime (0.1 to 10 keV) [9,23,27]. Fig. 1 shows the dose in-depth profiles of atoms incorporated using the energy regimes of implantation process. Each energy regime has a characteristic curve for ion penetration, but as can be seen the atomic areal density is very next to each other. According to the ion/target interactions, a lower energy source incorporates ions in near-subsurface regions, while a higher energy source increases the dose in-depth in a greater order of magnitude.

This work includes an alternative technology to the ion implantation techniques, which use implantation energies as high as 100 keV. From the industrial view, ion plating diversified (IPD) process would be more viable than conventional ion implantation, mainly, because of the short-time process, low-energy regime and capability of implantation in complex geometries by rotate-planetary system. Our process is able to be scaled up to industrial scale leading to yield high production rate with a relative simple arrangement at low-energy costs. Finally, this simple process to implant silver atoms (and other metals) at relative low energies may open new options for the development of metallic biomaterials with antibacterial action.

2. Experimental

2.1. Substrates

Samples of AISI 304 and 316L austenitic stainless steels, annealed and cold rolled were cut in the shape of squares with initial dimensions (20 × 20 × 0.05 mm). The chemical composition of stainless steels in accordance with the established specifications by ASTM A240 [30] and ASTM F139 [31] (C: 0.07, Mn: 2.00, S: 0.03, Si: 1.00, P: 0.045, S: 0.030, Cr: 19.00, Ni: 9.50, N: 0.10, balance Fe (wt.%)) and (C: 0.03, Mn: 2.00, S: 0.01, Si: 0.75, P: 0.025, S: 0.030, Cr: 18.00, Ni: 14.00, Mo: 2.5, N: 0.10, Cu: 0.5, balance Fe (wt.%)), respectively. The cleaning was conducted by immersion in acetone PA in ultrasound for 30 min. The plates were carefully dried and submitted to implantation process. Finished the treatment of surface samples were precisely cut into four parts (10 × 10 mm) and stored in vacuum desiccators.

2.2. Ion plating diversified equipment

The IPD process was accomplished in reactive low voltage ion plating-type equipment as shows in Fig. 2. This laboratory scale reactor was projected by researchers and contributors from *Laboratório de Engenharia de Superfície e Tratamentos Térmicos* (LESTT-UCS). The operational principle of IPD process is as following: (i) thermionic effect and electrons deflection, (ii) ionization and excitation, and (iii) ions acceleration. Succinctly, in stage I, thermionic electron are generated from a hot filament and deflected due a magnetic field (Lorentz force) leading to focus the primary electron beam toward the same center where silver pellets are located. The energy generated in the collision is sufficient to cause silver evaporation. In stage II, a secondary electron beam is generated by power source, freed by extraction system and directed to induce the formation of silver ions. Electrons induced collide with the residual gas atoms or silver atoms vaporized and form positively charged ions. A vacuum system provides fundamental guarantee for obtaining the ion beam, and a focusing system installed for the control of beam spreading. In stage III, positive ions will be accelerated by the electric field and implanted vertically into the negative potential surfaces. The main advantages of IPD process are the possibility of ion dose control, lower work temperature, short-time process, high degree of reproducibility, versatility of ion species and low-energy implantation.

Silver ions were implanted into one side of the plates. The vacuum in the reactor during implantations was kept at 1×10^{-3} Pa in both steels, and the base pressure was 1×10^{-4} Pa. The silver pellets evaporated in the process with purity of 99.9% provided by the company Kurt J. Lesker Company, United States. A piezoelectric sensor, located above the ionizer in the central region of the reactor, allowed controlling the amount of evaporated material. The ion electric current was measured by using a Faraday cup between 0 and 2000 μA on three tracks with insulation for 5 kV and reading accuracy ±5%. The reactor was cooled down with commercial nitrogen gas. Operating conditions IPD are described in Table 1. Under these process conditions, the regular silver doses for both the stainless steels were obtained.

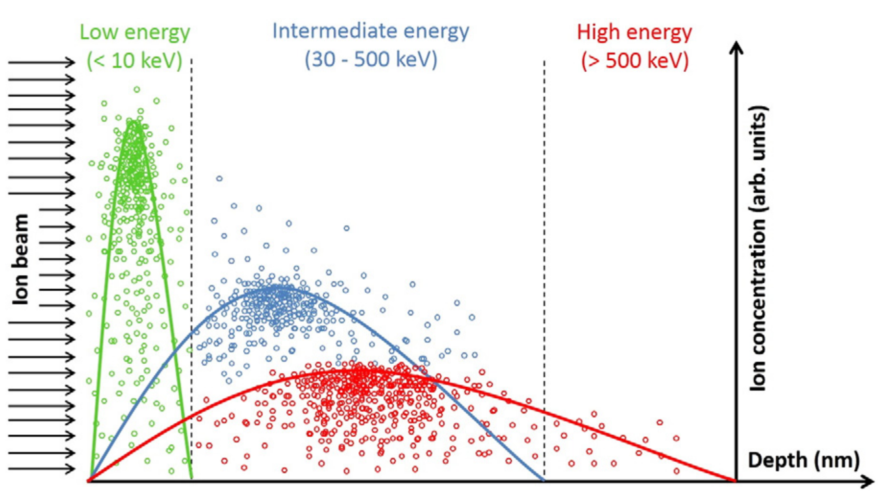

Fig. 1. Energy regimes of interaction ion/target surface for IPD.