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doi:10.1017/S1431927621010953

Microsc. Microanal. 27 (Suppl 1), 2021

Electron microscopy analysis of biofilms produced by *Staphylococcus aureus* exposed to UV-light on the surface of SnO_2 thin films

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Introduction

As an n – type wide band gap semiconductor (Eg = 3.6 eV) SnO_2 is one of the most intensively studied materials owing to its technological applications. The anomalous variation in the size and shape dependent properties of tin oxide nanoparticles makes it a promising candidate in optoelectronics, sensing, laser, solar cell, photocatalytic and biological applications. The photocatalyst material plays a crucial role to perform an effective photocatalytic reaction, and therefore its selection should be done carefully to fulfill both, appropriate electronic structure and reasonable energy of light for its photoactivation as the rate of the photocatalytic reaction is independent of the "active site". The antimicrobial efficiency of metal oxide nanoparticles depends on the particle size, presence of light, composition of aqueous medium used in assay etc. Electrostatic interactions are responsible for the attachment of nanoparticles to the bacteria. These interactions changes the integrity of cell membrane of bacteria and toxic free radicals are released which induce oxidative stress on bacteria [1]. Since antibiotic-resistant bacteria strains have become a serious health problem worldwide, it is a priority to develop new antimicrobial treatments that are not classified as antibiotics, and nanotechnology could be a valid alternative to revert this tendency [5].

Materials and Methods

Synthesis of SnO_2 thin films were performed by the spray pyrolysis method by addition of Tin chloride to an80ºC heated glass surface (slides). Thin films characterization was performed by X-Ray diffraction, SEM (JSM-5800LV) microscopy and EDS (results reported in previous publication)[2]. *Staphylococcus aureus* ATCC 29213 was stored in Brain-Heart infusion until use in antimicrobial assays. Before the assays, a bacterial solution containing $2x10^7$bacteria/ml was prepared in distilled water used in the inoculation of SnO2 films by adding a 10µl drop on the surface. Two groups of samples were then classified: a control group with no exposure to UV light, and a group exposed to 60 minutes to 254nm UV light. In order to allow biofilm formation, thin films were then stored 14 days at room temperature until SEM analysis.

Results

UV-light treatment was effective in eliminating *S. aureus* from the surface of the SnO_2 thin films thanks to the photocatalysis of this semiconductive material, this result is supported by previous studies that concluded that this kind of material has a good antimicrobial activity, especially when exposed to UV radiation [3, 4, 6]. Micrographs in Figure 1 shows the reduction of organic material when a comparison is made between the films without treatment (Fig. 1A) and after 60 minutes of exposition (Fig. 1B). As mentioned before, biofilms are a virulence factor that protects bacterial cells from adverse environments, and help pathogenic bacteria to spread and cause infections. Such structures are present only in control samples (Fig. 2), however this kind of cells agglomerates are no longer detectable after the UV treatment. These results suggest that photocatalytic materials are promising disinfection methods that could be applied to a wide variety of microorganisms.

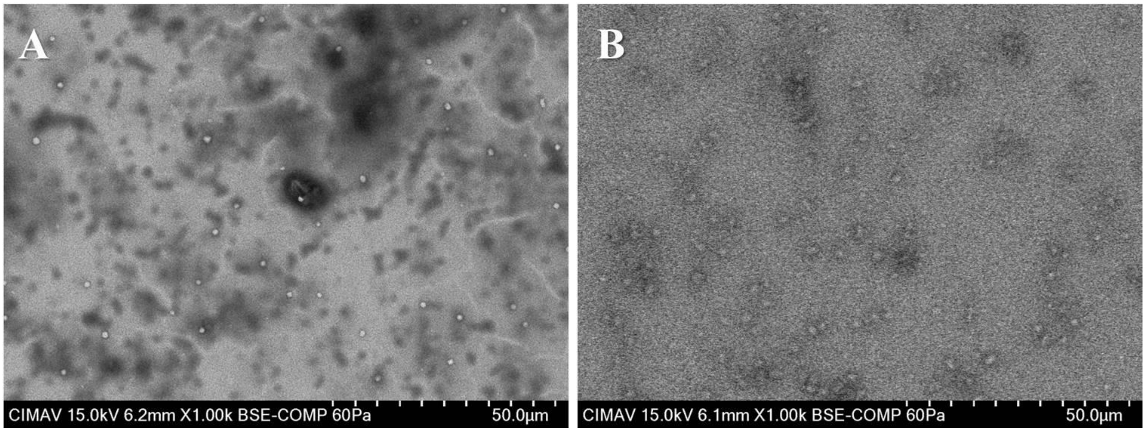

Figure 1. Micrographs at 1,000X using the Backscattered electrons technique to compare the presence of organic material on the surface of SnO_2 thin films inoculated with *S. aureus* before (A) and after (B) exposure to 60 minutes of UV light.

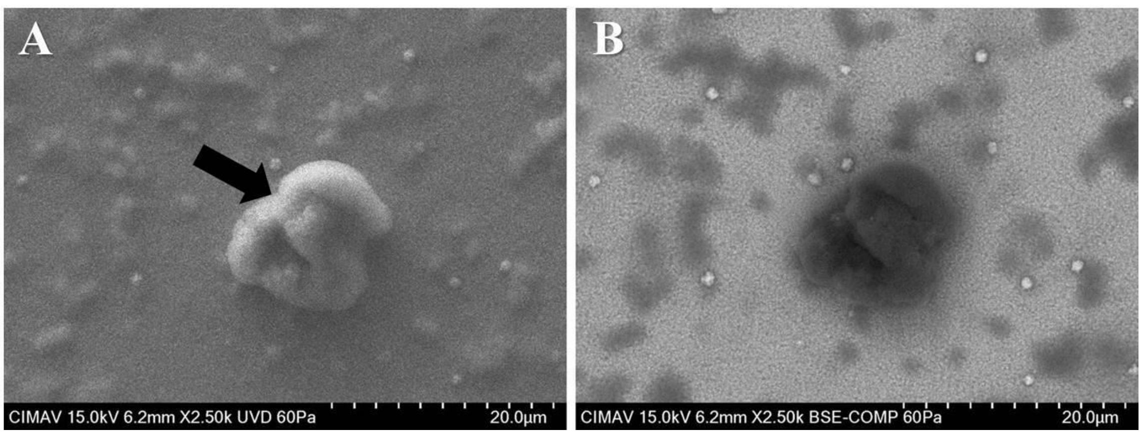

Figure 2. Micrographs at 2,500X magnifications of the surface of SnO_2 thin film inoculated with *S. aureus*. Figure 2A (secondary electrons) shows the morphology of a biofilm (Black arrow) of *S. aureus*. Figure 2B (Backscattered electrons) confirms the organic composition of the biofilm.

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