FLAME AEROSOL SYNTHESIS OF TIO_2 NANOPARTICLE COATINGS WITH ADVANCED ANTIBACTERIAL AND HYDROPHILIC PROPERTIES

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Abstract

In this work, the production of thin coating layers of TiO_2 nanoparticles by aerosol flame synthesis and direct thermophoretic deposition is reported. Three different flame reactor configurations were designed in order to study the effect of particle size on the performances of coating layers. The wetting behavior was investigated by water contact angle analysis, showing that titania coating layers are characterized by a high photoinduced hydrophilicity. Measurements of the inhibition of *Staphylococcus aureus* biofilm formation revealed a high antibacterial activity from TiO_2 nanoparticle films. Hydrophilic character and bactericidal effect are found to be mainly dependent on the dimension of primary particles composing the coating layers. The optimal synthesis conditions have been identified to produce a self-cleaning and self-disinfecting coating layer material, with a nearly superhydrophilicity and a high antibacterial activity, both activated by normal ordinary solar light radiation in standard room illumination conditions.

Introduction

Nanomaterials and their assemblage into functional devices and coatings are currently playing an increasing role in several fields, spanning from energy production and environment to biomedicine and biotechnology [1-2]. Flame synthesis of nanomaterials is characterized by a proven scalability, a fast processing time and an ease of manufacturing and collection [3]. In addition, nanostructured films and coatings of flame synthesized nanoparticles are produced in a single-step process by placing a cold substrate downstream the flame synthesis burner, on which nanoparticles are deposited by thermophoresis [4]. A relevant field of application of functional nanoparticle coatings is biomedicine. Nanomaterials characterized by an antimicrobial activity can be used as coatings with the aim to fight the proliferation of potentially pathogenic microorganisms as fungi and bacteria, since they possess the capability to inhibit microbial growth [5].

Among several antimicrobial nano-crystalline materials, titanium dioxide (TiO_2, titania) is the most harmless to environment and human health [6].
In this work, thin coatings of titania nanoparticles synthesized in flame were produced from a single step procedure by direct thermophoretic deposition on surfaces. Particle and coating properties were characterized using Differential Mobility Analyzer (DMA) and Raman spectroscopy. In addition, the hydrophilic/hydrophobic characteristics of the coatings were also investigated. Inhibition of biofilm formation was evaluated against Staphylococcus aureus bacterium as a function of nanoparticle size and coating morphology.

Experimental
TiO_2 nanoparticles were synthesized in an Aerosol Flame Synthesis (AFS) system, coupled to a thermophoretic deposition device made of a rotating circular aluminum disk. A detailed description of both the AFS system and the deposition system, as well as a scheme of the experimental set-up, can be found in previous works [7]. The flame reactor was obtained from premixed ethylene/air laminar flame doped with a solution of titanium tetraisopropoxide (TTIP, Aldrich, 97%) dissolved into ethanol (ACS reagent, ≥99.5%). In order to study the effect of particle dimension and coating morphology on the antimicrobial performances, three different flame reactor configurations, reported in Table 1, were investigated. Coatings were obtained using a disk rotating speed of 600 rpm, positioned at different height above the burner (HAB), corresponding to different particle residence times in the flame reactor. Circular substrates (diameter=16 mm, height=3 mm) made of aluminum alloy AA2024 were used.

Table 1. Summary of flame conditions.

Flame	TTIP, % mol	Φ	Disk HAB, cm
A	0.030	0.89	11
B	0.030	0.89	16
C	0.059	0.94	11

A dimensional characterization of the synthesized nanoparticles was made on-line by measuring number particle size distributions (PSDs) using a TapCon 3/150 Wien Type Differential Mobility Analyzer (DMA). Raman spectra were acquired using a Horiba XploRA Raman Microscope System (excitation wavelength λ=532 nm). The hydrophilic/hydrophobic properties were investigated by static contact angle measurements, following a previously described protocol [8].
Antimicrobial activity was investigated by testing the inhibition of biofilm formation of a Gram positive bacterium, *Staphylococcus aureus* (ATCC 6538), under ordinary light radiation in standard room illumination conditions. Bacteria cells (108 cellule/mL) were suspended in Mueller-Hinton Broth (MHB), aliquoted in a 12-wells plate (1.5 ml/well) containing the aluminum substrates and incubated

overnight at 37 °C. Aluminum substrates were moved in a new 12-well plate and washed three times with phosphate-buffered saline (PBS) to remove non adherent cells. SEM images of the samples were then acquired using a Carl Zeiss EVO MA 10 microscope with a secondary electron detector (Carl Zeiss SMT Ltd., Cambridge, UK). Finally, the biofilm mass was quantified by counting the number of bacterial colonies from SEM images using the image processing software ImageJ®.

Results and Discussion

The number distributions of particle diameter measured by DMA are shown in Fig. 1 (left panel), together with their best fit obtained by a lognormal distribution function or a sum of two lognormal distributions. From the fitting procedure, the mean particle diameter was found to increase from $\langle D_p \rangle = 2.6$ nm for Flame A, to $\langle D_p \rangle = 3.1$ nm for Flame B, due to the increase in particle residence time in the flames. On the other hand, the increase of both TTIP concentration and particle residence time in Flame C condition resulted in a bi-modal PSD with the first mode characterized by $\langle D_p \rangle = 2.4$ nm, while the second mode is characterized by $\langle D_p \rangle = 10.5$ nm. Fig. 1 (right panel) reports the normalized size distributions in mass. It can be assumed that the prevailing mass contribution to the TiO_2 coatings on the substrates is given by particles with a mean dimension of 4.5 nm (Flame A), 7 nm (Flame B) and 18 nm (Flame C).

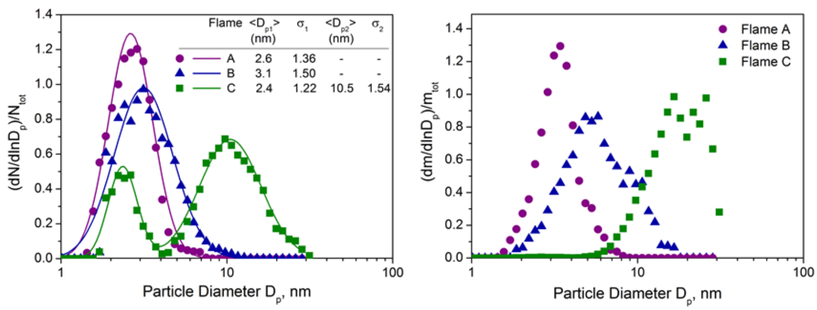

Figure 1. Left panel: Normalized number PSDs, $(dN/d\ln Dp)/N_{tot}$; symbols are experimental DMA data, lines are best lognormal fits of data. Right panel: Normalized size distributions in mass, $(dm/d\ln Dp)/m_{tot}$.

The phase composition of as-deposited coating layers was determined using Raman spectroscopy. The acquired spectra are presented in Fig. 2, showing the presence of five Raman scattering peaks, corresponding to the Raman bands assigned to the allowed modes of anatase phase TiO_2 [9], thus confirming that coatings were made of pure anatase particles. In this study, flame reactors were operated in fuel-lean conditions in order to promote the formation of anatase (the most photoactive phase of titania), which is favored in oxygen-rich environments [10].

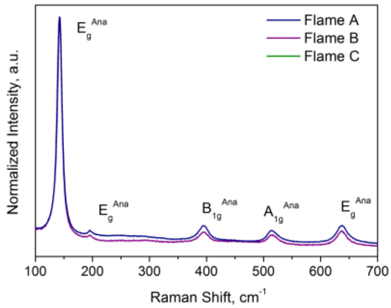

Figure 2. Raman spectra of TiO_2 nanoparticle coating layers.

The wettability of the coatings was investigated by water contact angle analysis and the results are reported in Fig. 3. Measurements performed under ordinary light radiation in standard room illumination conditions indicate that all the three coating layers were able to decrease the contact angle with respect to the bare aluminum substrate (62±3°). Particularly, the coatings produced in Flame A and Flame B possess a nearly super-hydrophilic behavior, with θ of about 10±3° and 10±3° respectively, while the angle measured for Flame C (33±3°) is larger. The high hydrophilicity of TiO_2 thin films has been demonstrated to be mainly caused by a photoinduced process [11]. This was confirmed by measuring the contact angles on deactivated TiO_2 samples, obtained by placing for 70 hours the coated substrates in the dark (Fig. 3, red bars). The values of θ without UV-visible radiation increased up to about 50° for all the samples.

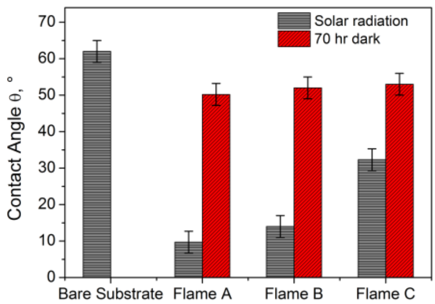

Figure 3. Water contact angles measured under ordinary room light (gray bars) and after 70 hours of dark storage (red bars).

Antimicrobial activity of nano-TiO_2 layers was characterized by evaluating the extent of *Staphylococcus aureus* biofilm formation by SEM analysis of the samples exposed to bacteria colonies. Obtained SEM images are reported in Fig. 4. The bare aluminum substrate, which represents the positive control, showed the presence of a stable and mature biofilm of *S. aureus* cells. On the other hand, it is possible to observe a clear decrease of the number of adherent *S. aureus* cells on

TiO_2-coated substrates. All the coating layers are characterized by a remarkable antibacterial activity under ordinary room light, which is higher for Flame B and Flame C coatings with respect to Flame A coating.

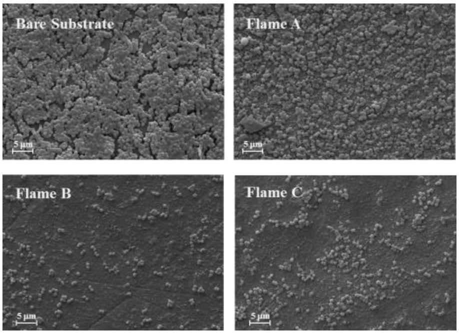

Figure 4. SEM microphotographs of *Staphylococcus aureus* biofilm on uncoated aluminum substrates and TiO_2 coated aluminum substrates.

From SEM images, the highest efficacy against *S. aureus* biofilm was measured on Flame B and Flame C coating layers, which were able to inhibit 82% and 80% of biofilm formation respectively, while *S. aureus* inhibition on Flame A coating was measured to be about 54%. Those results are reported in terms of percentage of biofilm formation relative to the positive control, i.e., the bare aluminum substrate, on which 100% of bacterium biofilm is formed. The measured trend of the antimicrobial activity is likely to be related to the size of particles composing the coating layers, suggesting the existence of an optimal size range for the maximum antibacterial efficiency [12]. However, further work is needed to isolate the effect of primary particle size from the effect of film properties on both hydrophilicity behavior and antimicrobial activity in our coating layers.

Conclusions

The use of a highly controllable and tunable technique for the production of a nanostructured TiO_2 coating film on aluminum substrates has been reported. This method, based on the combination of aerosol flame synthesis and direct termophoretic deposition, provides a fine control over particle size and crystalline phase, as well as film morphology. Nano-titania layers showed a high hydrophilic character and high antimicrobial activity against *Staphylococcus aureus* biofilm formation under ordinary light radiation in standard room illumination conditions. Both hydrophilicity and antimicrobial activity have been seen to depend on the dimension of primary particles composing the coating layers. Flame B coating layers possess the highest bactericidal activity, together with an almost superhydrophilic character, both photoactivated even under ordinary light radiation in standard room illumination conditions. The combination of such properties, taking place simultaneously on the same surface, widen the application as an excellent self-cleaning and self-disinfecting coating materials for the sanitization of environmentally contaminated surfaces in indoor and outdoor buildings [13].

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