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Antipathogen nanostructured coating for air filters

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Abstract

Indoor air quality has great impact on health and wellbeing of the occupants. Although modern air filtration systems assure high air quality, however the filtration media itself needs to be protected from microbial contamination. Low maintenance and dirt accumulation along with humid conditions in air ventilation systems create a suitable environment for the growth of microorganisms on the surfaces. Microbial proliferation undermines both the quality of the filtered air and the filtration apparatus. Contaminated air directly affects occupant's health, comfort and productivity, with an augmented risk of infectious diseases. The aim of this work was the development of an air filter with innovative nanostructured coating, able to confer anti-pathogenic functionalities without altering filtration performance of the filter. The filter was accomplished by depositing a nanostructured composite silver/silica coating on the surface of a glass fibres based nonwoven filter. This developed air filter completely inhibited the growth of microorganisms even when exposed to bioaerosol of high bacterial concentration. The filter showed good antibacterial performance under practical conditions for extended period spanning one month. The innovative nanocomposite coating imparts long lasting antimicrobial properties to improve the performance of the filtration systems enabling them to perform better with minimal maintenance efforts.

Keywords: Air filter; antipathogen; coatings; nanostructure

1. Introduction

People in the world spends on average about 90% of their time indoor [1, 2]. Indoor air quality, hence, directly affects occupant health, comfort and productivity and the exposure to air contaminants for

long period is associated with adverse human health effects [3, 4]. In fact, airborne microorganisms or bioaerosols such as bacteria, fungi, viruses, molds and pollen are the main carriers of allergic reaction and infectious diseases. More widespread health diseases include allergy from exposure to indoor pollutants (particularly those associated with building dampness and molds), colds and other infectious diseases transmitted through the air due to elevated indoor pollutant levels as well as other indoor environmental conditions; more serious health impacts, resulting from poor air quality, include Legionnaires' Disease [5, 6]. Current air filters in Heating, Ventilating and Air Conditioning systems (HVAC) act as particulate's capture and separation with a very high retention capability for bioaerosols, reducing the circulation of high number of microorganisms in the air [7]. Air filter, however, represents the most susceptible part to microbial colonization. Bioaerosol grows on the filter media itself and maintain their vitality because of dirt accumulation in HVAC systems, inadequate levels of particle filtration, poor filter maintenance, and problems with cooling coil condensate or other moisture sources. In the long term, people present in the rooms inhale air microbial contaminants, proliferated and then released from the HVAC system into the flowing air [7]. In this way, the risk of serious air quality problems drastically increases. In addition, it is known that microorganisms provoke corrosion of the metallic and polymeric portion of the filtration system [8].

Different but inefficient cleaning methods and disinfectants as ultraviolet germicide irradiations, photocatalytic oxidation and air ozonolysis methods have been developed [9, 10]. In the last year, various new strategies and approaches to prevent and overcome microbial growth on air filter, have been proposed using silver compounds and nanoparticles [9]. In general, silver is the most widely employed antimicrobial metal due its well-known and broad-spectrum anti-pathogen activity. Ionic or metallic silver has been well studied in terms of antibacterial and antifungal properties; although there is still some debate on the exact mechanism of interaction with host cells [11]. In particular, silver nanoparticles (Ag-NPs) act with multifaceted mechanisms [12], which include oxidative stress induction, metal ion release, and non-oxidative processes. Silver ions can bind to the bacteria cell wall, break it and aggregate in the cytoplasm. The gradual release of free silver ions from Ag-NPs solution inhibits the bacterial cells proliferation. Silver-based coatings, deposited on polyurethane foam by means of *in situ* photo-reduction [13] and on polypropylene filters adding silver nitrate after the production cycle [14], significantly reduced the viability of several bacterial and fungal strains. Ko et al. produced monodispersed Ag-NPs decorated silica particles for synergic antibacterial activity against Gram positive and Gram negative bacteria in the air filtration unit, demonstrating a stability of the solution of Ag-NP and SiO₂ up to six months with 99% of antibacterial efficiencies for both the tested bacteria [15].

The aim of this work is the deposition and development of an antipathogen nanostructured coating for air filter, following a procedure patented by the authors [16, 17]. The innovative nanostructured coating, deposited via co-sputtering, is composed of a silica matrix embedding antipathogen silver nanoclusters [18]. Silver nanoclusters release ions in a gradual and control manner suitable for affecting bacteria, but they are not toxic for human external exposure [19] and they are not dispersed into the surrounding environment thanks to the silica matrix [20, 21]. Test of permeation into skin for 24h demonstrated an ion release of about 1.5 $\mu\text{g/L}$ when the limit of toxicity is less than 500 $\mu\text{g/L}$ [20-22]. In addition, the coating is able to withstand thermal treatment up to 450 °C without changing its antibacterial properties [19]. The sputtered silver nanocluster/silica composite coating is an effective and versatile solution able to confer antimicrobial properties to various materials (polymers, metals, textiles) for different applications. In fact, the authors have already developed and studied the nanostructured composite coating for several applications with encouraging results, from biomedical implants[23, 24] to aerospace structures [20, 25], mobile phone [26], natural and technical textiles [20-22]. In this work, two procedures for analysing the antipathogen effect of the coating were developed, using an experimental setup and protocols for bacterial contamination test in order to simulate working conditions of HVAC system in the laboratory. In addition, the silver nanoclusters/silica composite coating deposited on air filter was tested in a real and working conditioner for 30 days, demonstrating the efficacy also against bioaerosols, naturally present in a laboratory.

2. Methods

2.1 Coating deposition and characterization

A silver nanocluster doped silica matrix coating was deposited on glass fibre based air filters, supplied by Sagicofim Spa, by a radio frequency (RF) co-sputtering process. The company provided some properties of the material: the filter weight is $78 \pm 6 \text{ g/m}^2$, the penetration of Di-Ethyl-Hexyl-Sebacat (DEHS) is 0.005% and water repellence is about 500 mm. The coating has a composite structure, made of a silica matrix with silver nanoclusters uniformly embedded in it. More details about this coating and its deposition method are reported in the previous papers of these authors [18, 19]. To summarize, two cathodes were used in the sputtering equipment (Microcoat™ MS450), a silver one (Sigma-Aldrich™ 99.99% purity) and a silica one (Franco Corradi S.r.l.™ 99.9% purity). The co-sputtering process was previously optimized to obtain approximately a 300 nm thick coatings, in about 80 minutes, in pure argon atmosphere applying a power 200 W in RF mode for silica, 1 W in DC for silver and operating pressure of 5.5 dPa.

Scanning electron microscopy (Field Emission Scanning Electron Microscope FESEM, QUANTA INSPECT 200, Zeiss SUPRATM 40™ with Energy Dispersive Spectroscopy (EDS, EDAX PV 9900™) was used to observe and analyse the coated and uncoated filter morphology and composition. Silver release tests were done to quantify the ionic silver amount released in water from the coated filter up to 15 days. Coated filters (1cm² area) were immersed into 25 ml of MilliQ water. Silver ion content was analyzed after 3 h, 24 h, and 3, 7 and 14 days of immersion using silver photometer (Hanna Instruments™). The test was carried out in triplicate for each measurement.

2.2 Bacterial contamination test

The antipathogen behaviour of the coated filters (diameter 4 cm) was evaluated by means of a bacterial contamination test against two different bacterial strains, *Staphylococcus epidermidis* (Gram +, ATCC14990) and *Escherichia coli* (Gram -, ATCC8739). The test was performed in a controlled manner, by using a standard bacterial suspension applied for different exposure times in an experimental setup built to simulate air ventilation system conditions. For all the tests, the standard suspension was prepared starting from a bacterial culture grown on Nutrient agar for 24 hours at 37 °C, and then, suitably diluted in order to obtain a value of Optical Density, of a sample measured at a wavelength of 600 nm (OD₆₂₀) between 0.8 and 1. The apparatus for the bacterial contamination (Fig. 1) consisted of a bioaerosol generator (TOPAS, ATM 220) and a filter holder. This device is a simplification of what reported in [14]. In our case, compressed air with adjustable airflow (pressure at the instrument = 3 bar) flows through the aerosol generator, producing the bioaerosol from the bacterial suspension. Bioaerosols enter the filtration column and pollute the filter inserted into the filter holder. During a preliminary test, the bacterial viability was checked in order to determine a suitable time for the filter contamination tests with TOPAS apparatus. For this purpose, the standard bacterial suspension of *S. epidermidis* was nebulized on Mueller Hinton agar plates for three different times (1, 3, 7 min). After 24h of incubation at 37 °C, the colonies forming units (CFU) were observed. Based on the obtained results, two qualitative contamination tests were developed: short time and intermediate time exposure. *In the first short test*, a bioaerosol with the bacterial suspension containing *S. epidermidis* or *E. coli* was nebulised on coated filter for 3.5 or 7 min. Filter was then removed from filter holder and the bioaerosol exposed face was deposited on the surface of a Mueller-Hinton agar plate. After an incubation at 37°C up to 48h, bacterial growth was evaluated around and below the filter surface. Each test was performed in triplicate and a filter without coating was used as control.

The *intermediate test* consisted in the exposition of coated filter to a standard bacterial suspension (*S. epidermidis* or *E. coli*) bioaerosol: in this case, four exposition cycles (7 min for the 1st cycle, 3.5

min for the subsequent one) were performed for a total contamination time of 17.5 minutes. Contaminated filters were incubated as previously described for *short test*.

At the end of both kind of tests, a *replica plating test* was performed: the filters (reversed face) were deposited on the surface of a new Mueller-Hinton agar plate and incubate them 2 hours at 37 ° C. After that, the filters were removed from the agar surfaces, the agar plates were incubated again up to 48 h at 37 °C and then the bacterial growth on agar plate was evaluated.

2.3 Bacterial contamination in real air conditioner

Two coated and uncoated filters were placed into a real air conditioner, working in one of our laboratories, for 30 days. Both filters were then incubated on a Mueller Hinton agar plate up to 24h at 37°C and the bacterial growth was observed.

3. Results

3.1 Coating deposition and characterization

The air filter (Fig. 2a) used in this work was composed of randomly oriented glass-fibres, as visible in the SEM micrographs in Fig. 2b. The silver nanocluster/silica composite coating was effectively deposited by co-sputtering technique on the glass-fibre filters. Fig. 3 shows the glass fibre morphology before and after coating. The uncoated glass-fibres appeared very smooth (Fig. 3a, b) whereas the coated fibres presented a globular morphology typical of these coatings, as reported in the previous papers [20-22]. It is worth noting that the coating does not affect the filtration functionality, since the deposited layer is highly conformal and does not close or obstruct pores of the filter. Silica matrix completely embedded silver nanoclusters, which were not visible on the fibres. However, EDS analysis (Fig. 4) confirmed the presence of Ag into the coated filters only. Regarding the other detected elements, Si and O, were both belonging to fibres and coatings, plus K, Na, Al, Ca and Ba, belonging to the glass fibre original composition.

Even if silver is a well-studied antimicrobial element, it was important to verify and quantify the amount of ions released into the surrounding environment by this coating. In this paper, the coating was intended for air filtration, i.e. a humid environment, but it was not supposed to work immersed in water or other water based solution. In this respect, the leaching test in water was a very severe one with respect to the final application.

Fig. 5 shows the cumulative silver ion amount continuously released in water at RT up to 14 days. Silver ions were released in a progressive manner with a steeper curve slope during the first 3 days, indicating a faster release of ions during the first period of immersion. After 14 days, the amount of silver ions reached the value of 0.3 ppm. This registered maximum amount after 14 days was within

the range of antimicrobial behaviour (0.1 ppb), but well below the toxic (10 ppm) one for human cells [27, 28].

This gradual release in water was different from what reported in [28]: in that case, the sputtered Ag doped – TiO₂ coating on stainless steel was immersed into phosphate-buffered saline solution (PBS) and the maximum release of silver ions was obtained in the first 24 h, then it gradually decreased. This behaviour can be suitable for implantable biomaterials, whereas in this work, the progressive release of silver ions is more suitable for an air filter, which needs a continuous antipathogenic effect, hopefully lasting the filter entire life.

In addition, the test in Fig. 5 was stopped after 14 days, but the curve did not reach a plateau yet, thus opening the possibility of an even longer antipathogen activity.

3.2 Bacterial contamination test

In order to define suitable protocols for bacterial contamination test on the coated air filters, a test of bacterial viability was preliminary performed. The standardized solution of *S. epidermidis* was nebulised on a Mueller Hinton agar plates for 1, 3 and 7 minutes. Fig. 6 shows the results obtained after the incubation at 37°C for 24h. The test demonstrated that the TOPAS apparatus did not affect the viability of the tested bacteria also after 7 minutes of bioaerosol generation. Moreover, 1 minute was enough to obtain a high bacterial contamination on the agar plate.

On the basis of the obtained results, the standardized bacterial suspension of *S. epidermidis* or *E.coli* was nebulised on both the coated and uncoated filters for the short time test (3.5 and 7 minutes). After the contamination, both the filters were incubated on a Muller Hinton agar plates and the results are shown in Figure 7 and 8. It is possible to observe that this coating completely inhibits both *S. epidermidis* (Fig.7 b, d) and *E.coli* (Fig. 8 b, d) growth on filters, whereas bacterial colonies are well visible on the uncoated ones (Fig. 7 a, c and Fig. 8 a, c). This indicates that the bacteria sprayed on the surface of coated filters were completely killed by the coating. It is important to underline that the coating demonstrated a significant bactericidal effect with a bacterial reduction of 100% on coated air filters. The brown colour, noticeable of the coated filter, is typical of this coating [20-22]. The presence of silver, in the form of nanoclusters, promotes a scattering effect, giving a consequent surface dark color, prevalent with respect to the effect of surface plasmon resonance as discussed in [18].

This coating antipathogenic behaviour was confirmed by means of replica plating test. Fig. 9 and 10 show the agar plates which have been in contact with the previously contaminated coated and uncoated filters. Bacterial growth was evident only for uncoated control filters.

The same results were obtained also for test performed for a longer time, as reported in Fig. 11. As for the previous tests, a bacterial growth of *S. epidermis* or *E.coli* was **formed** only on the plates, which were put in contact with the uncoated and contaminated filters.

3.3 Bacterial contamination in real air conditioner

Finally, the antipathogen effect of uncoated and coated filters was tested also after exposition towards bioaerosol, naturally present in a laboratory environment. Both the filters were positioned into a working air conditioner in one of our laboratory for a period of 30 days and then analysed as previously described (i.e. incubation at 37°C for 24h). Fig. 12 reports the photographs of the obtained results, which demonstrate the efficiency of this coating in a real working condition: different colonies of (**not yet defined**) airborne bacteria and fungi are visible on the uncoated filter, whereas they are not present on the coated one. This result are also in accordance with the data obtained during the ion release test reported in Fig. 5: silver ions were progressively released in water, but the curve did not reach a plateau after 14 days. This may signify that the effectiveness of this coating *in air* could be much longer than what measured in water: after 30 days, no viable bacteria proliferated on the coated filter. A longer test of at least 6 months is ongoing for further demonstrating the duration of antipathogen effect for longer period, for a better comparison with the commercial filters.

4. Conclusions

The antipathogen silver nanoclusters/silica composite coating was successfully deposited on the glass-fibre air filter by means of co-sputtering technique. The nanostructured antipathogen coating well covered the surface of glass fibres without closing the porosity of the filtering media. In this way, the filtration functionality was not affected. Silver ions were gradually released in water up to 14 days, reaching a value well below the silver toxicity threshold but higher than the minimum amount for antimicrobial effect. The progressive ion release made the nanostructured coating suitable for devices and applications needing a prolonged and continuous antipathogen behaviour as that of the air filtration. A proper experimental setup was developed in order to verify the antipathogen efficiency simulating the working conditions of air filter in a controlled manner. The nanostructured coating completely prevented microorganism growth on the air filter in the case of both gram positive and gram negative bacteria. A test in a real and working HVAC system showed the efficacy of the coating also for a period of 30 days. Promising results in terms of duration and prolonged exposition time will be estimated, leading to positive impacts on health, safety and wellbeing of the entire population. In fact, the antipathogen nanostructured coating will find an enlarged range of application in the health sector, making a decisive contribution to the containment of biological risk and thus

implementing the established prevention and safety procedures. Moreover, it could be used in the realization of further protective kits such as respiratory devices and masks improving the safety of health personnel. Its use could be extended also in very crowded places and at risk of spreading diseases such as public transport, kindergartens, offices, gyms and sports facilities.

Journal Pre-proofs

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Journal Pre-proofs

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Figure Captions

Fig.1. Experimental setup for bacterial contamination test of filters: (a) schematic representation and (b) equipment

Fig. 2. Glass fibre based filter used in this work, before coating: (a) macro-photograph of filter (b) SEM micrograph of the filter showing the glass fibre random structure.

Fig.3. SEM micrograph of the glass fibre based air filter used in this study: (a, b) uncoated and (c, d) coated filters, showing the typical morphology of the Ag/SiO₂ sputtered coating [20, 22, 23].

Fig. 4. EDS analysis of (a) uncoated and (b) coated filter, showing the presence of silver in the coated one

Fig. 5. Silver release in water of an air filter coated with the antipathogen coating.

Fig. 6. Mueller Hinton agar plate, contaminated by aerosolized *S. epidermidis* for (a) 1 minute, (b) 3 minutes and (c) 7 minutes, after incubation at 37 °C for 24 h.

Fig. 7. Short test of bacterial contamination indicating performance of filters against *S. epidermidis* at an exposure time of 3.5 min (a) uncoated and (b) coated glass fibre filter; of 7 min (c) uncoated and coated glass fibre filter.

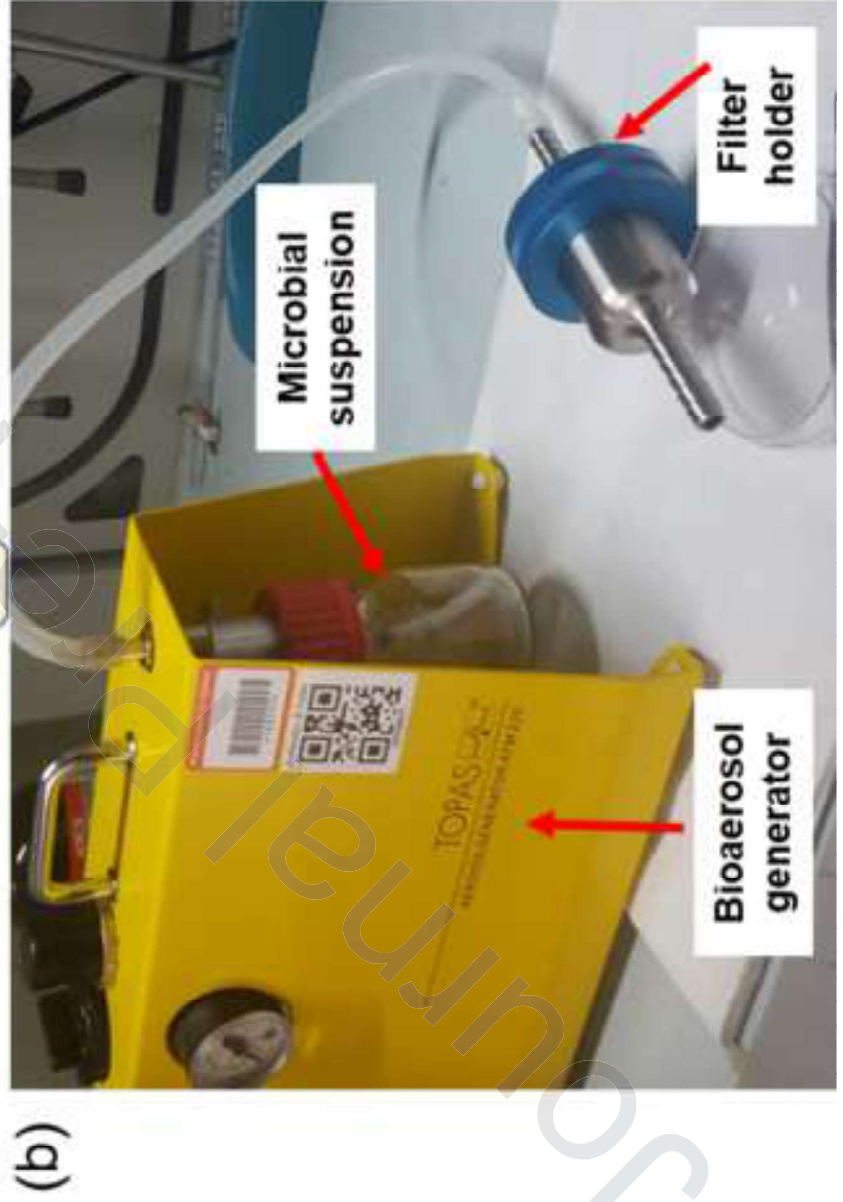
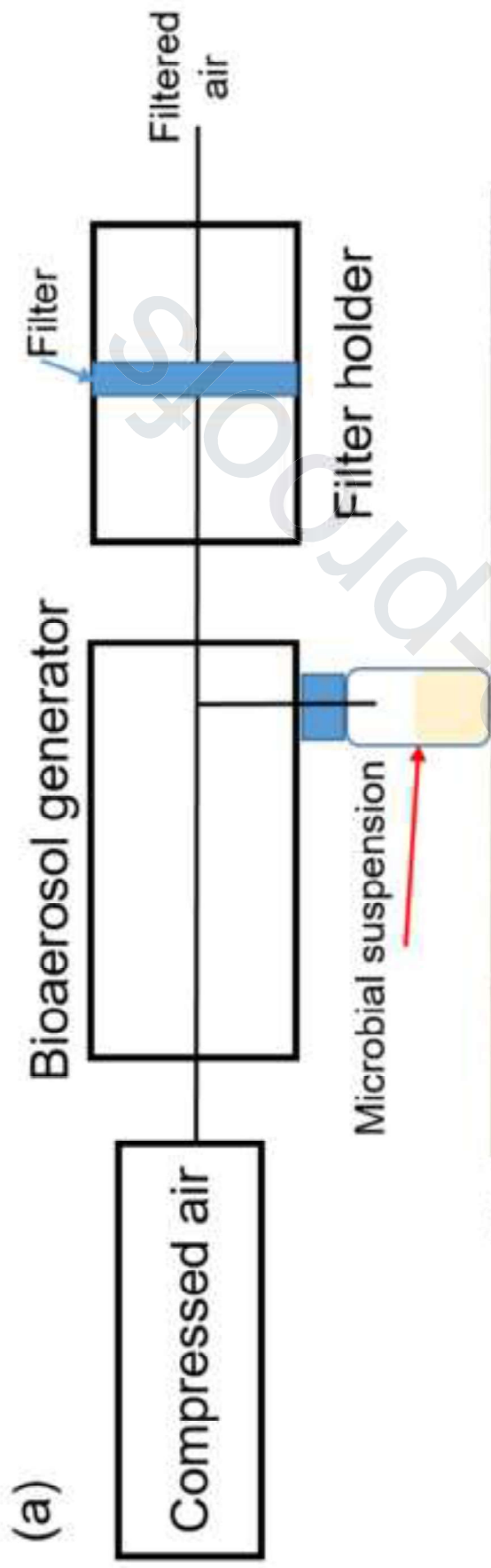
Fig. 8. Short test of bacterial contamination indicating performance of filters against *E. coli* at an exposure time of 3.5 min (a) uncoated and (b) coated glass fibre filter; of 7 min (c) uncoated and coated glass fibre filter.

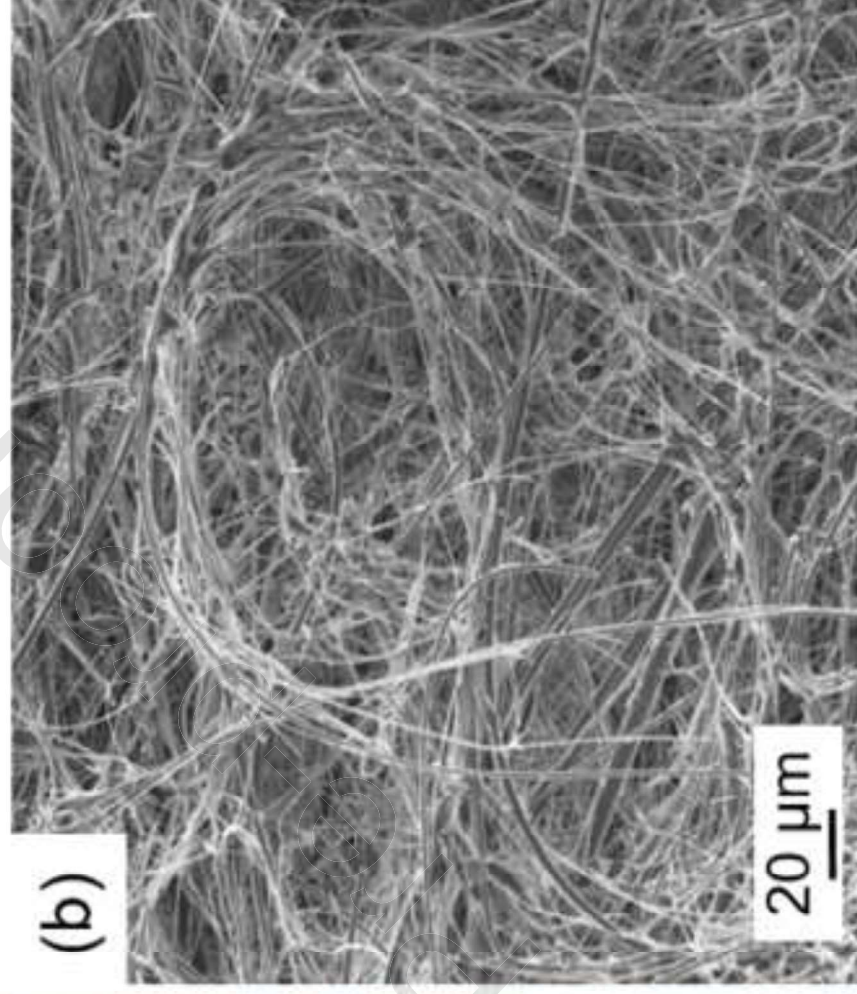
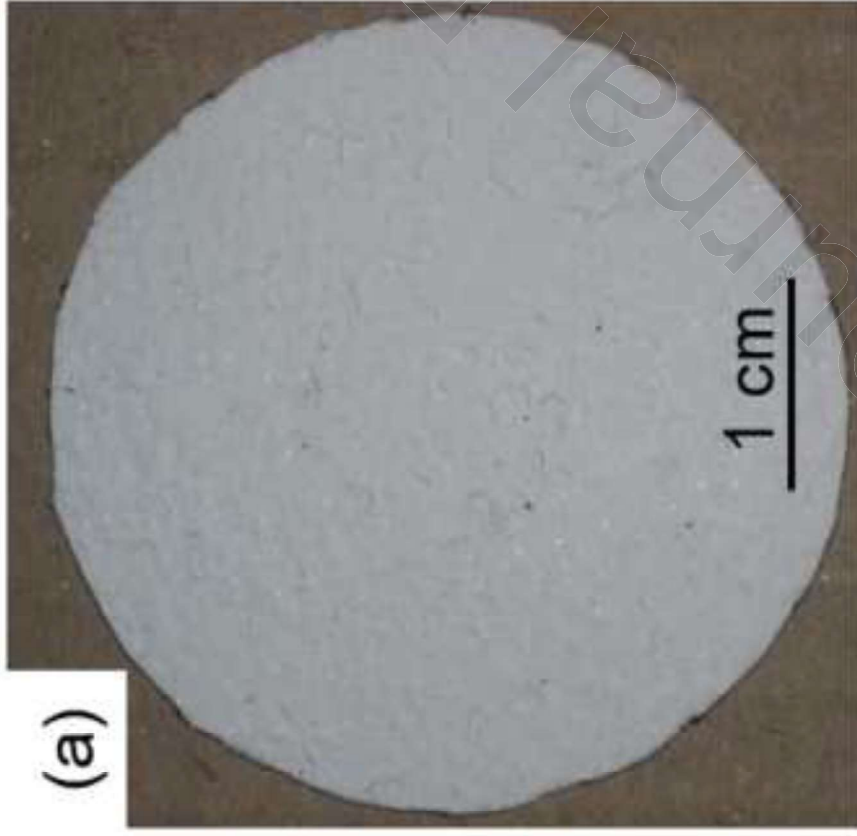
Fig. 9. Replica plating test of uncoated and coated filters towards *S.epidermis* at different exposure times: 3.5 min (a, b) and 7 min (c and d). The agar plates in contact with coated air filters do not present any bacterial growth (b and d), whereas a relevant bacterial proliferation is visible on the agar plates which were in contact with the uncoated filters (a and c).

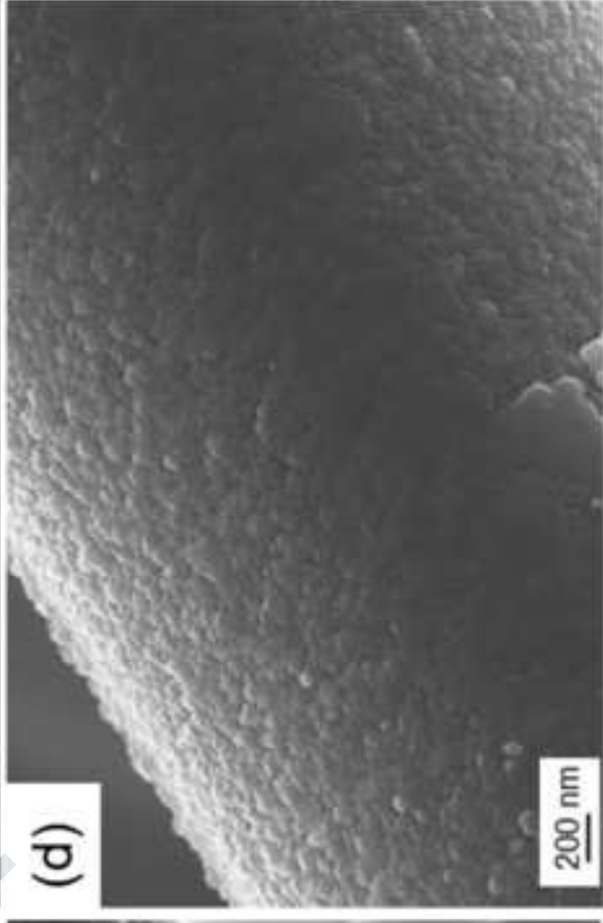
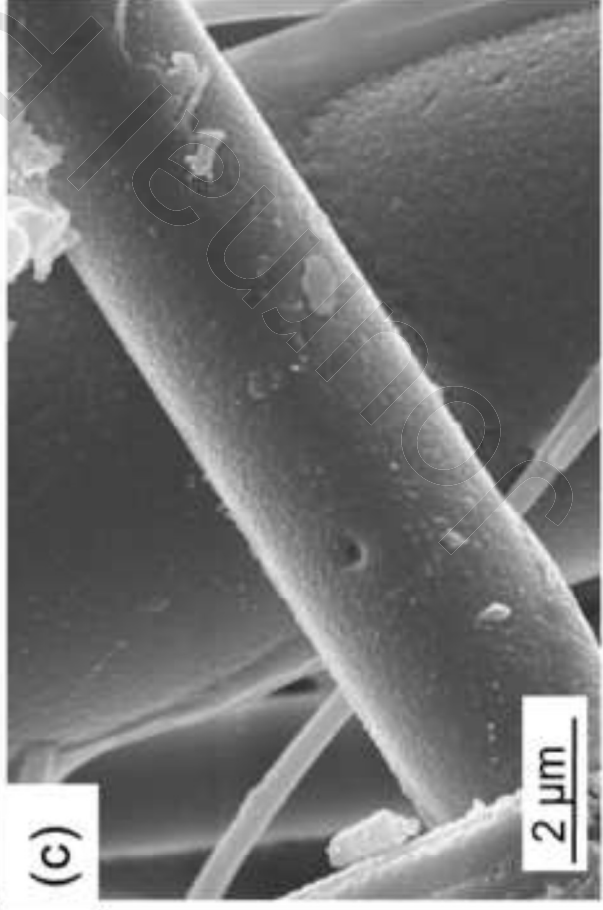
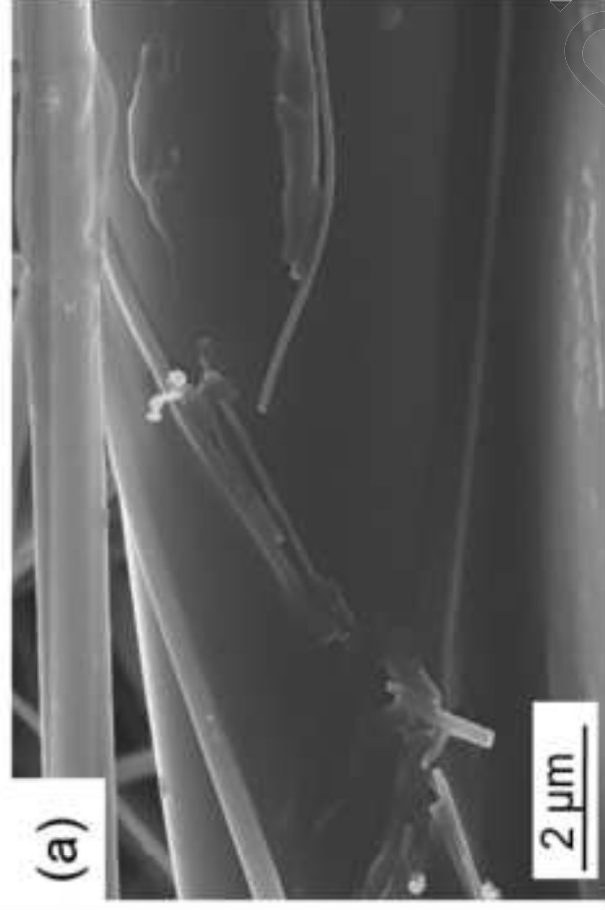
Fig. 10. Replica plating test of uncoated and coated filters towards *E. coli* at different exposure times: 3.5 min (a, b) and 7 min (c and d). The agar plates in contact with coated air filters do not present any bacterial growth (b and d), whereas a relevant bacterial proliferation is visible on the agar plates which were in contact with the uncoated filters (a and c).

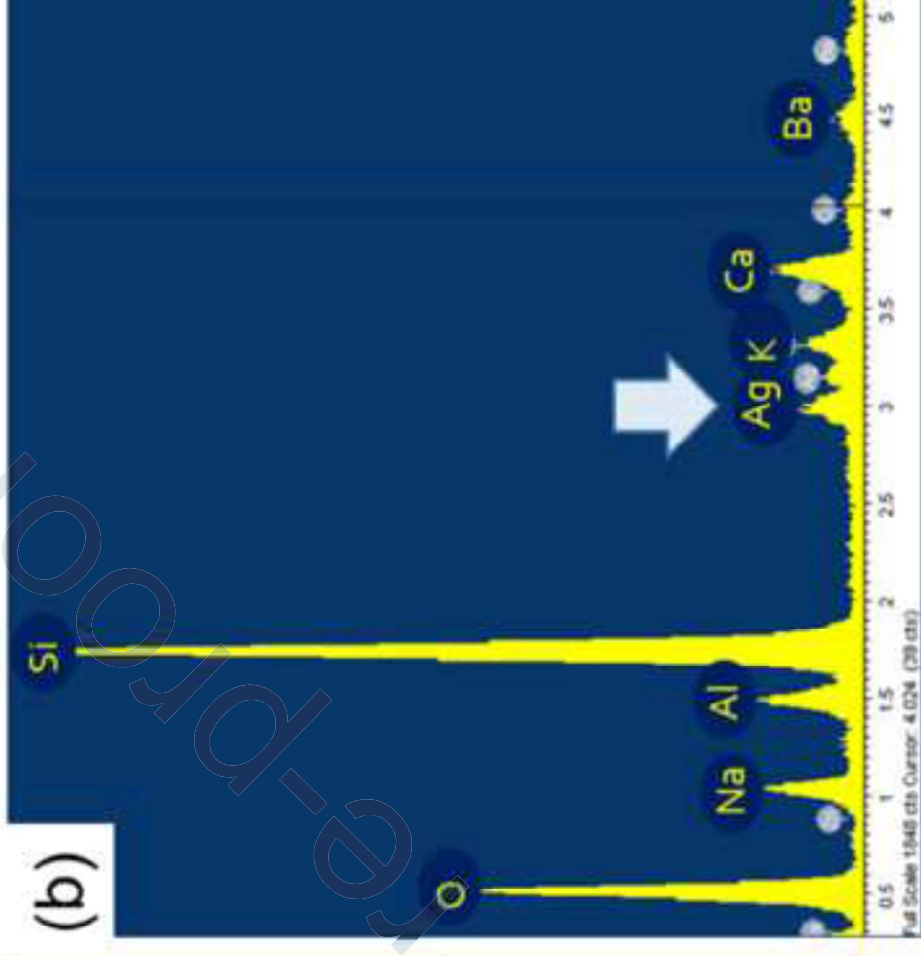
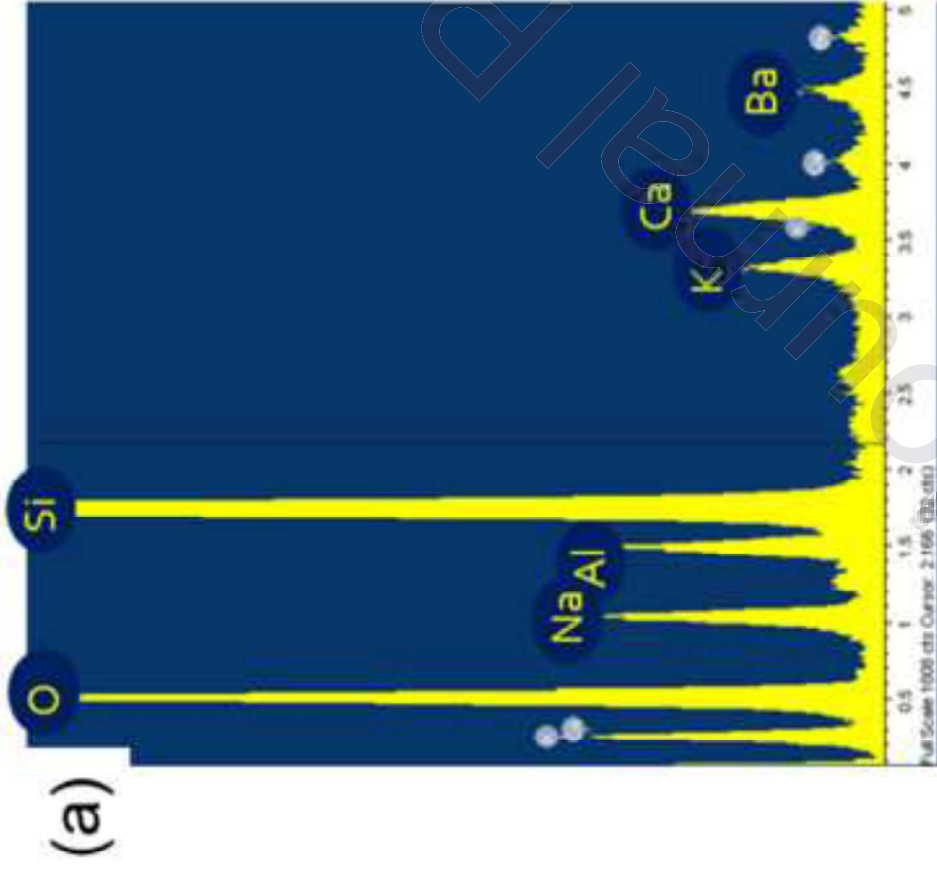
Fig 11. Intermediate test of bacterial contamination indicating performance of filters against *S. epidermidis* and *E. coli* after 17.5 min of effective exposure time: uncoated glass fibre filter (a and e) and the relative plating reply test (b and f); coated glass fibre filter (c and g) and the relative replica plating test (d and h).

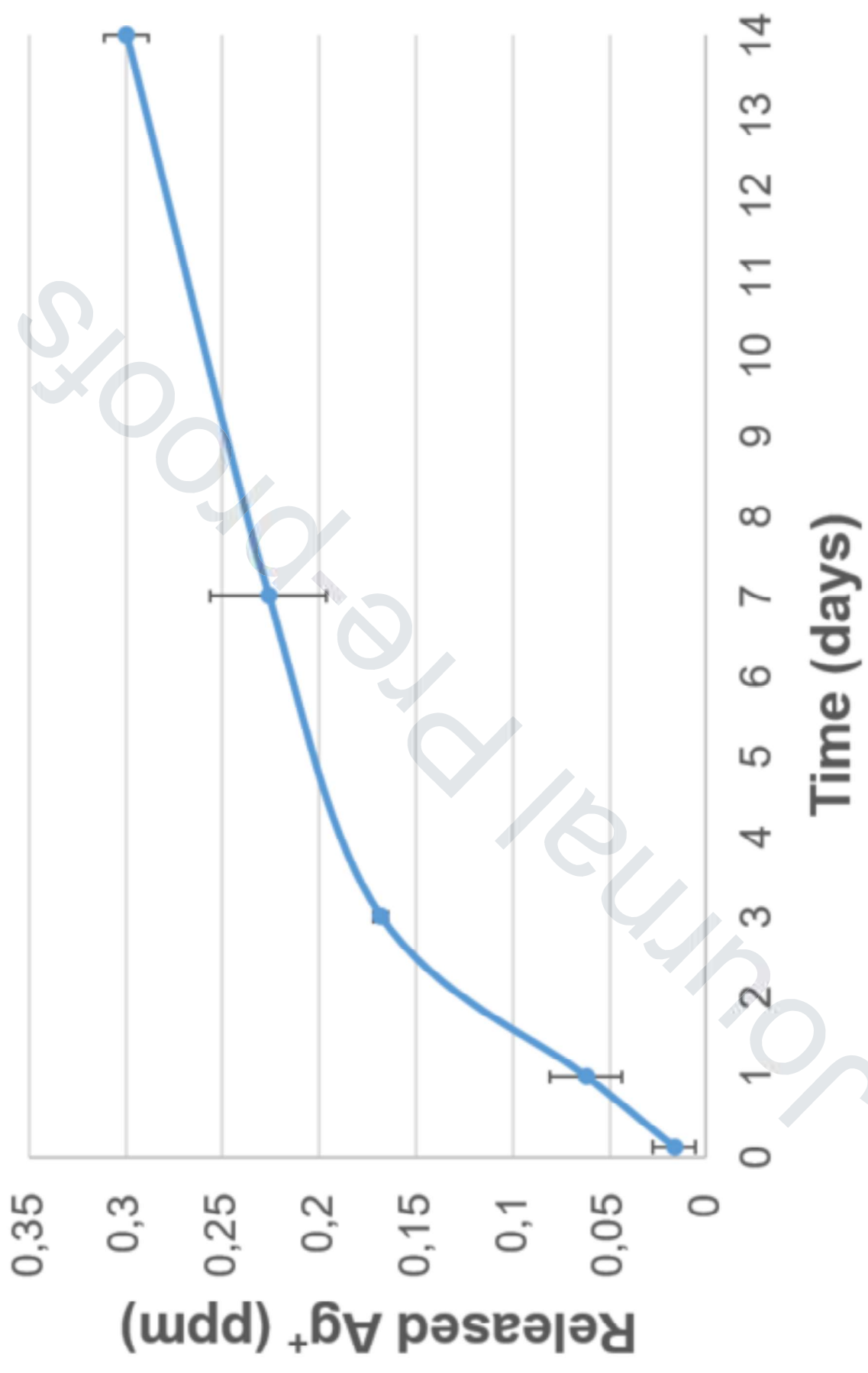
Fig. 12. Bacterial contamination in a working air conditioner filter after 30 days: (a) bacterial growth on uncoated filter; (b) coated filter without bacterial growth.

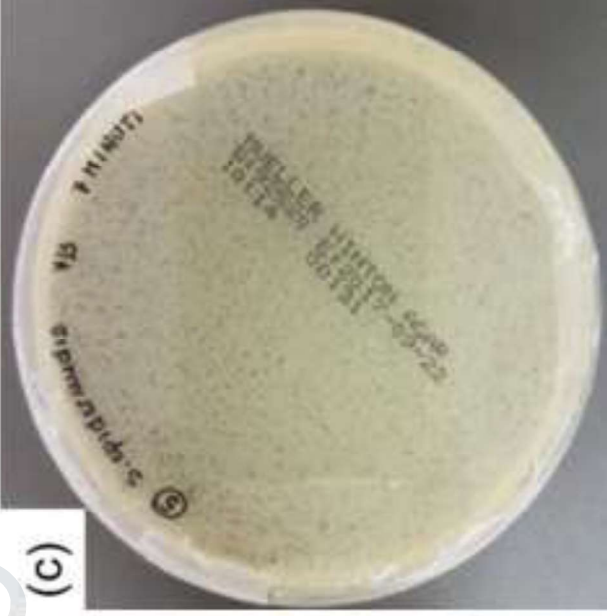
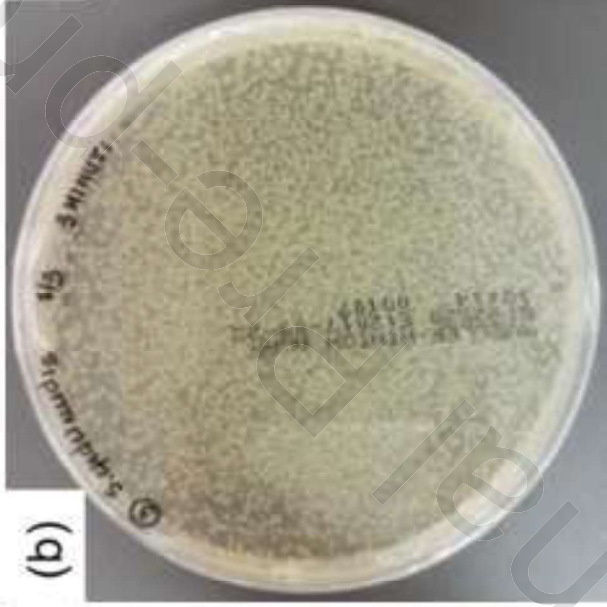
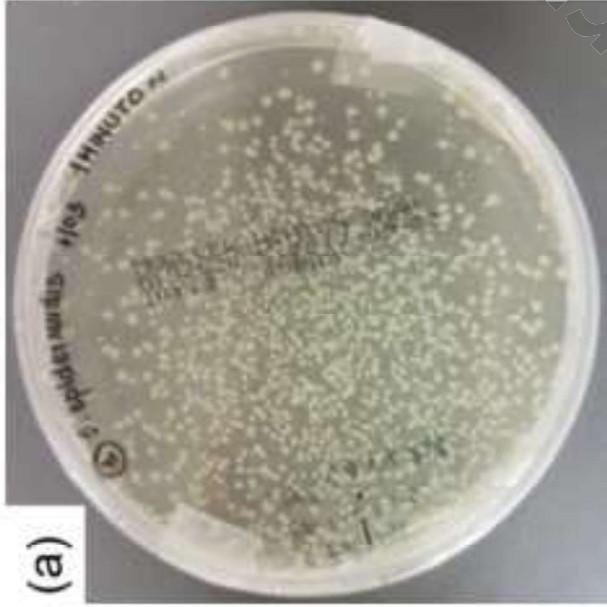




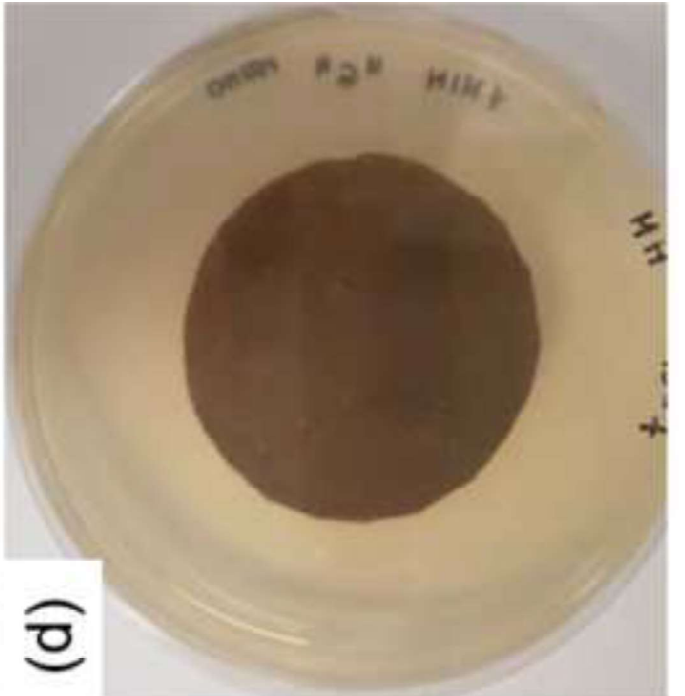
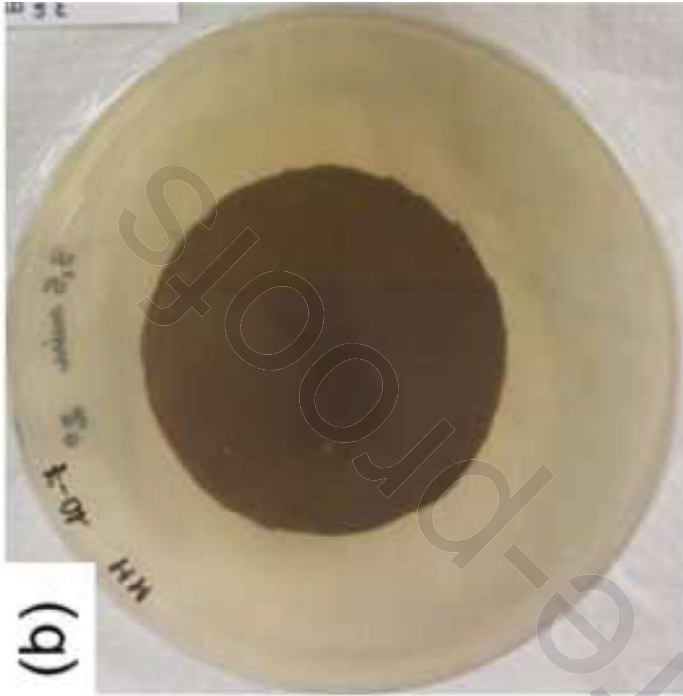
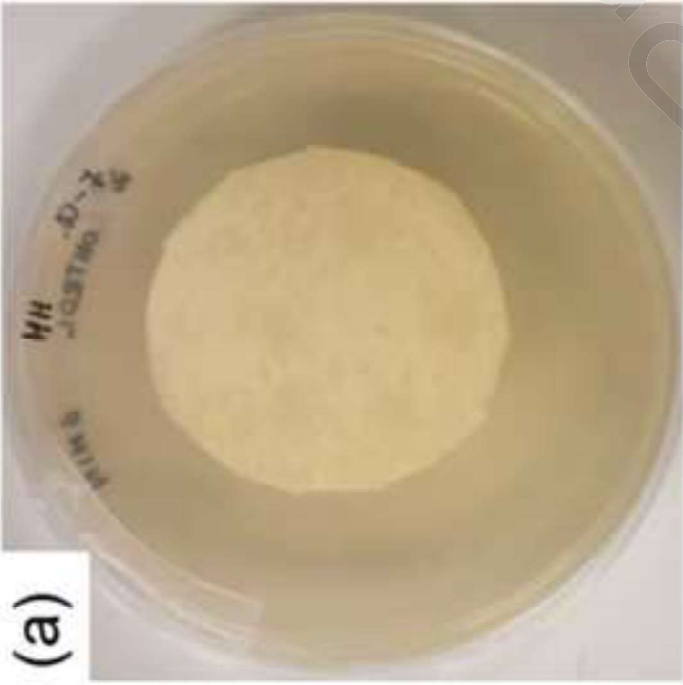


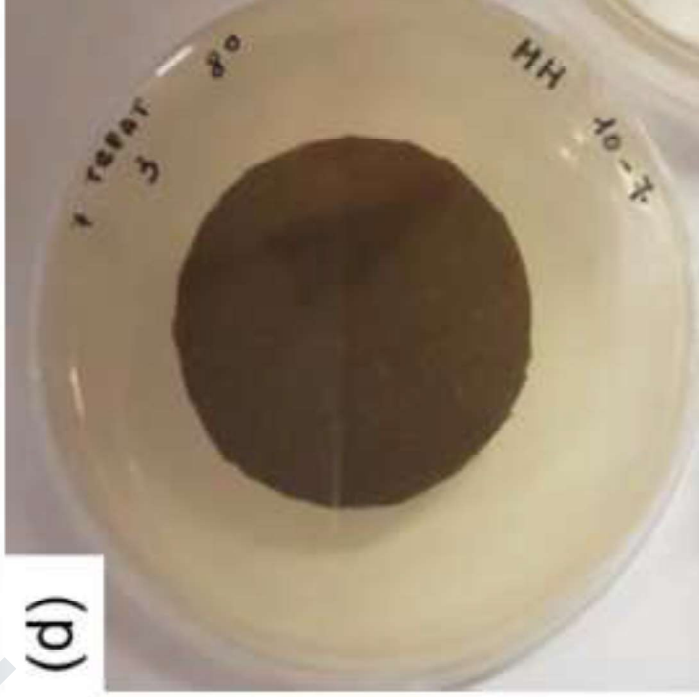
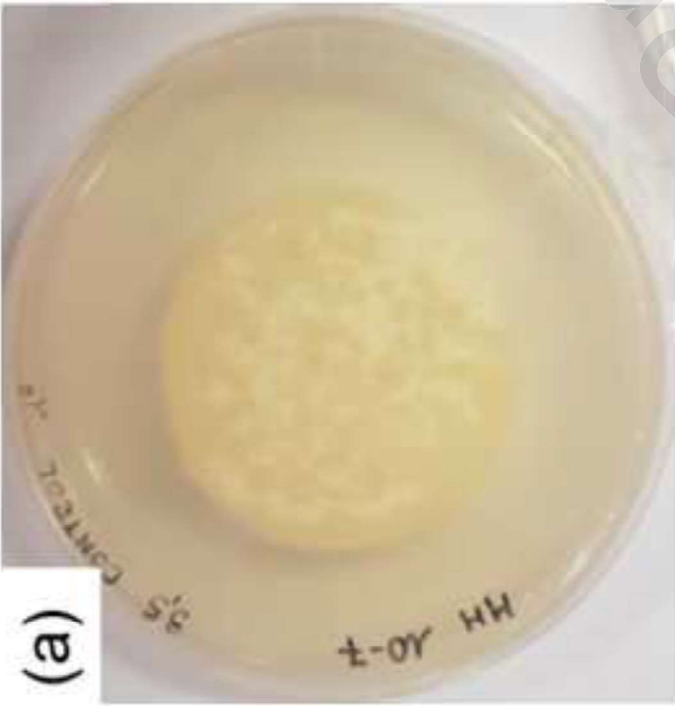


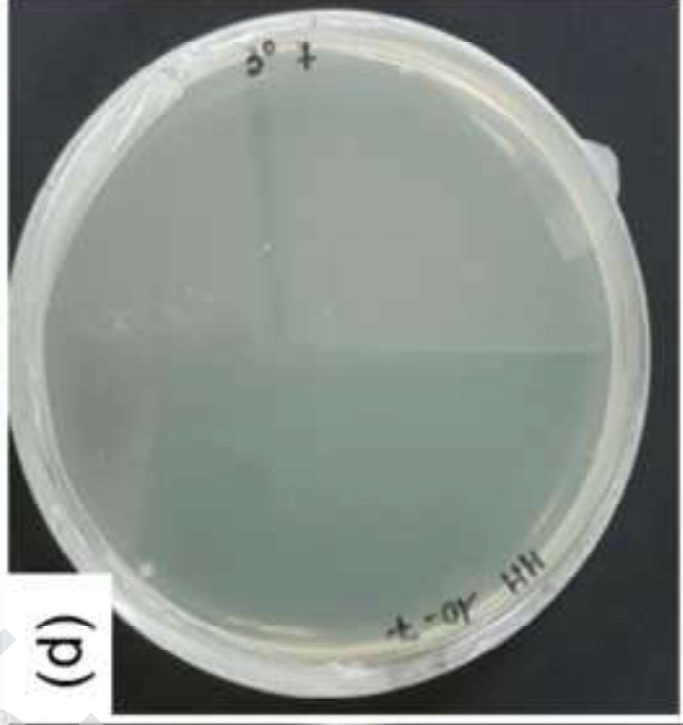
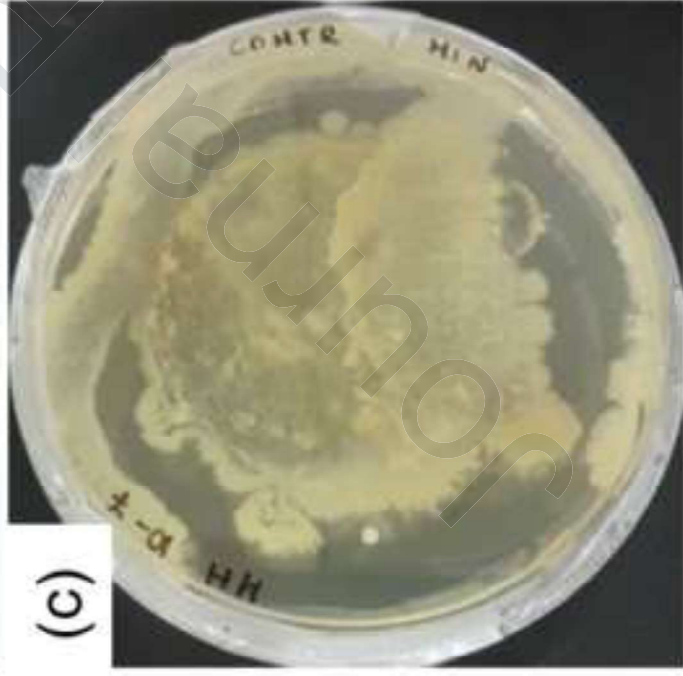
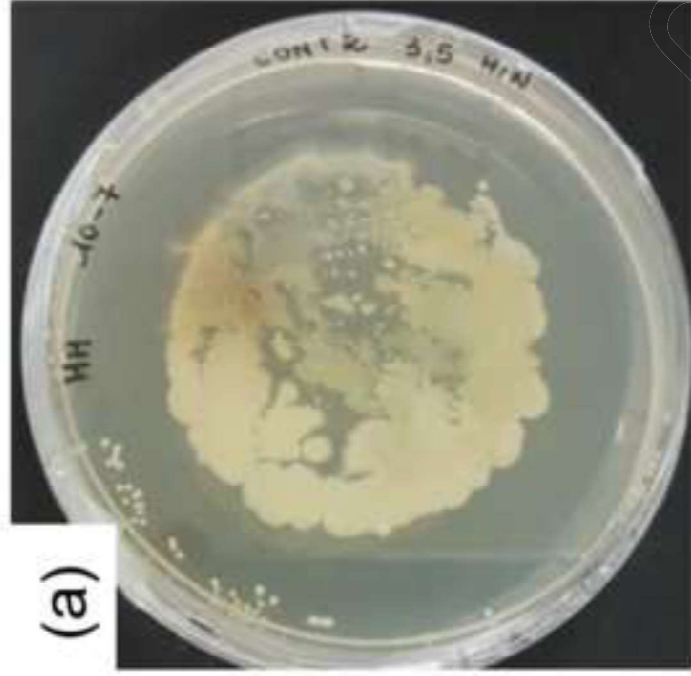


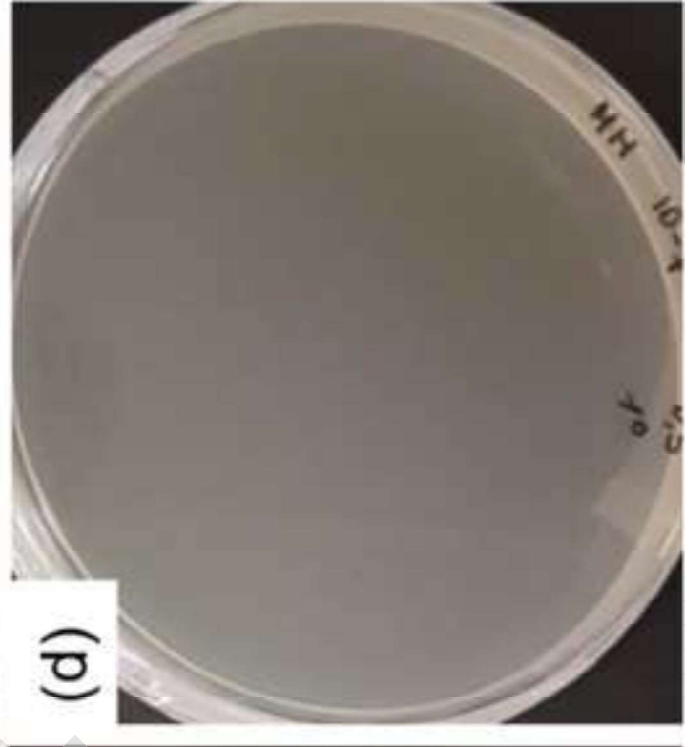


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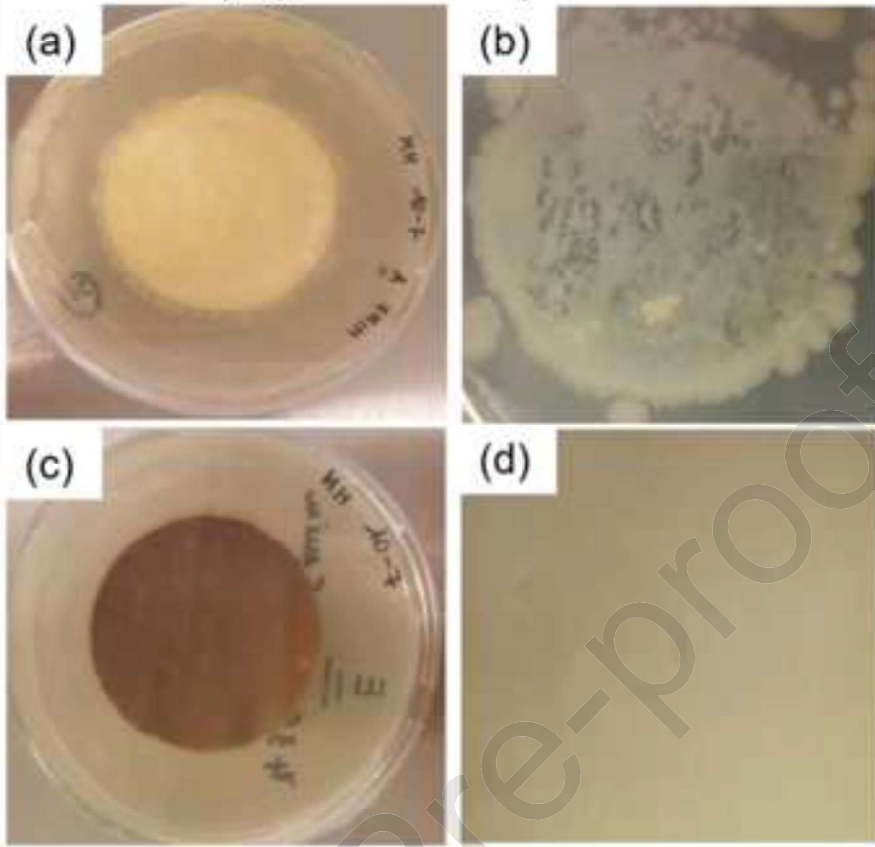




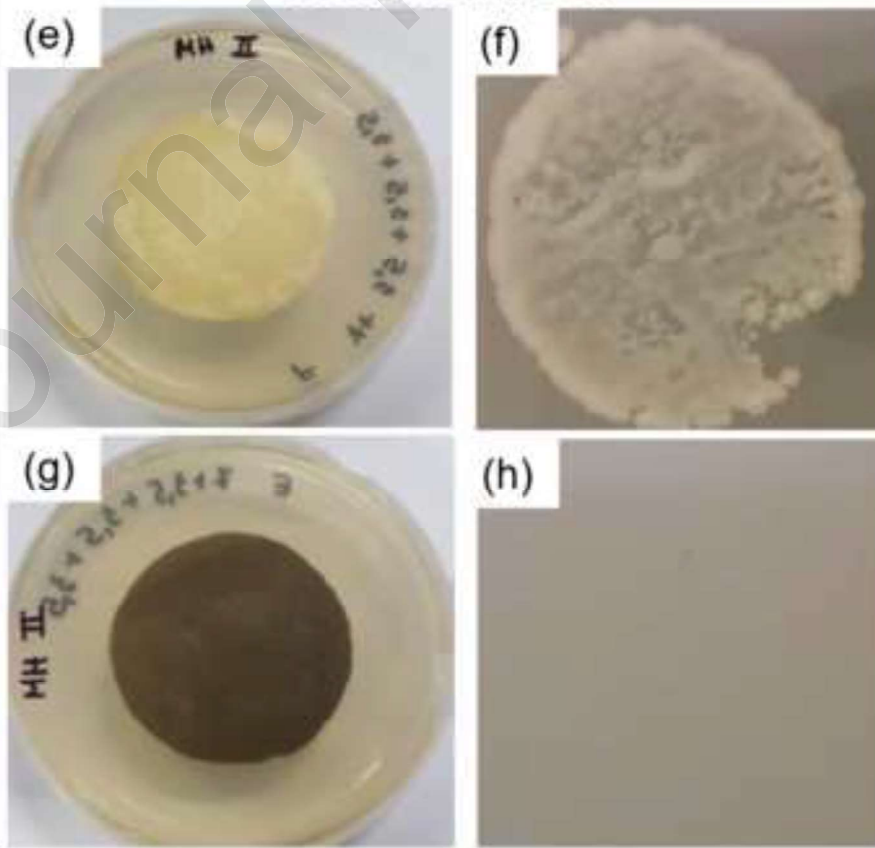


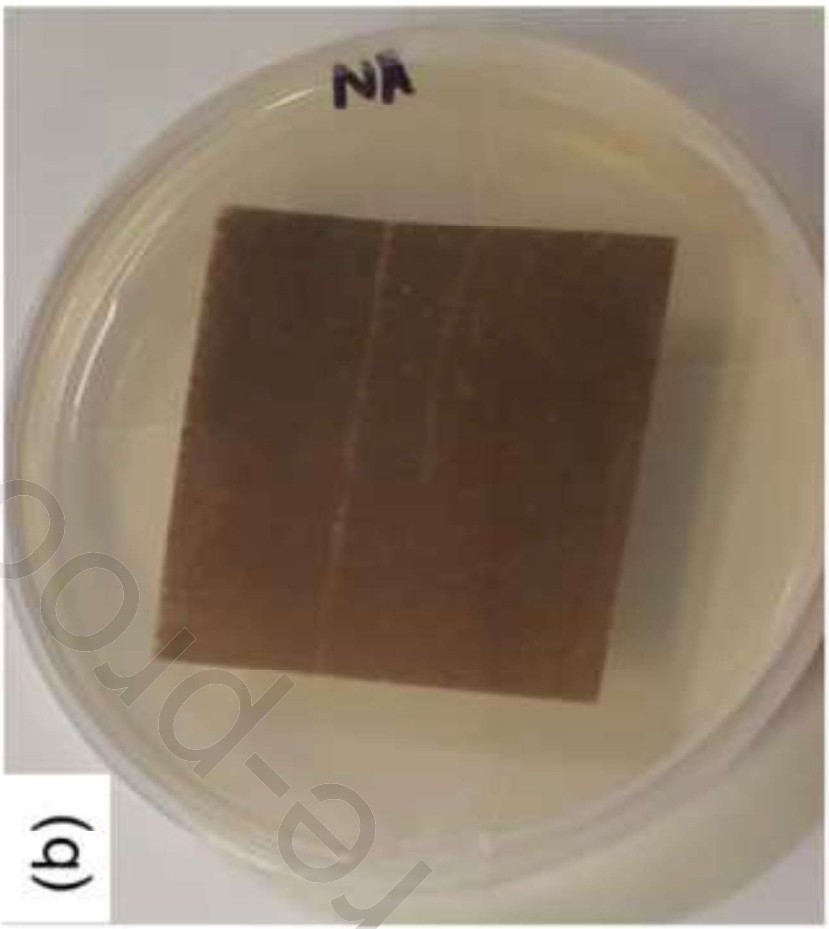


Staphylococcus epidermidis



Escherichia coli





Research highlights

- Silver nanocluster/silica composite coating was deposited on air filter
- A co-sputtering technique was used for the coating deposition
- A proper experimental setup was developed for verifying the antipathogen efficiency
- The nanostructured coating completely prevented biofilm formation on the air filter

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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