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Comparison of Methods for Evaluation of the Bactericidal Activity of Copper-Sputtered Surfaces against Methicillin-Resistant *Staphylococcus aureus*

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Bacteria can survive on hospital textiles and surfaces, from which they can be disseminated, representing a source of health care-associated infections (HCAIs). Surfaces containing copper (Cu), which is known for its bactericidal properties, could be an efficient way to lower the burden of potential pathogens. The antimicrobial activity of Cu-sputtered polyester surfaces, obtained by direct-current magnetron sputtering (DCMS), against methicillin-resistant *Staphylococcus aureus* (MRSA) was tested. The Cu-polyester microstructure was characterized by high-resolution transmission electron microscopy to determine the microstructure of the Cu nanoparticles and by profilometry to assess the thickness of the layers. Sputtering at 300 mA for 160 s led to a Cu film thickness of 20 nm (100 Cu layers) containing 0.209% (wt/wt) polyester. The viability of MRSA strain ATCC 43300 on Cu-sputtered polyester was evaluated by four methods: (i) mechanical detachment, (ii) microcalorimetry, (iii) direct transfer onto plates, and (iv) stereomicroscopy. The low efficacy of mechanical detachment impeded bacterial viability estimations. Microcalorimetry provided only semiquantitative results. Direct transfer onto plates and stereomicroscopy seemed to be the most suitable methods to evaluate the bacterial inactivation potential of Cu-sputtered polyester surfaces, since they presented the least experimental bias. Cu-polyester samples sputtered for 160 s by DCMS were further tested against 10 clinical MRSA isolates and showed a high level of bactericidal activity, with a 4-$\log_{10}$ reduction in the initial MRSA load (10^6 CFU) within 1 h. Cu-sputtered polyester surfaces might be of use to prevent the transmission of HCAI pathogens.

Health care-associated infections (HCAIs) are acquired by patients during the process of care in a hospital or other health care facility. Annually, in Europe, 5 million people acquire HCAIs, causing 135,000 deaths and representing an economic burden of $16 to 30 billion (http://helics.univ-lyon1.fr/helicshome.htm). In the United States, around 2 million patients suffer from a HCAI, from which about 90,000 will die (12). HCAIs represent a major problem that is still underestimated in developed countries and even more so in developing countries (1). HCAIs can be acquired by pathogens present on commonly touched surfaces, such as door handles, table tops, hospital gowns, and bed sheets, where bacteria remain alive for long periods of time and can be spread by hand contact. Despite important hand washing campaigns such as those steered by the World Health Organization (6, 19), infection rates remain high, and additional measures should be taken. A measure complementary to hand washing might be the reduction of the bacterial burden in the hospital setting. A decrease in the number of people infected in hospitals was observed previously when the bacterial concentration was partially reduced in hospital rooms (7). In that context, the use of antimicrobial surfaces is currently being investigated, and an increasing number of surfaces have been created to prevent bacterial colonization (4, 10, 20). Surfaces made of metals like silver (Ag) or copper (Cu) have shown a high level of efficiency in the killing of bacteria (16, 25). When used as additives, Cu^{2+} ions showed the best compromise between antibacterial efficiency and cytotoxicity compared with aluminum (Al^{3+}), cobalt (Co^{2+}), zinc (Zn^{2+}), and mercury (Hg^{2+}) when tested against *Staphylococcus epidermidis* and fibroblast cells of the connective tissue (11). The mechanism of the bactericidal activity of Cu is still poorly understood. The bactericidal effect of Cu is due to the release of Cu^{2+} ions into the surroundings, since Cu^0 itself is not bactericidal (21). It is believed that the antimicrobial activity of Cu is multifactorial and involves mechanisms such as the generation of reactive oxygen species, which induce oxidative injuries to bacterial DNA; cell wall or iron-sulfur cluster-containing enzymes; as well as the binding of free Cu ions to proteins which alter their function (9, 15, 17, 20, 24, 26). All mechanisms work together, ultimately leading to cell death.

Previous work indicated that Cu-sputtered films exhibited a better bacterial inactivation activity than TiO_2- or Ag-sputtered films under the same experimental conditions (2, 18). Currently, there is no well-established method to assess the bactericidal activity of this type of surface (14, 18).

HCAIs can be caused by a number of bacterial pathogens, such as vancomycin-resistant enterococci, *Clostridium difficile*, or *Pseudomonas aeruginosa*, but methicillin-resistant *Staphylococcus aureus* (MRSA) currently represents one of the most important challenges of infection control. This study focuses on the comparison of four different methods to assess the viability of an MRSA strain (ATCC 43300) on Cu-sputtered polyesters: mechanical detachment, microcalorimetry, direct transfer onto plates, and stereomi-

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croscopy. Furthermore, the bactericidal activity of Cu-sputtered polyester against 10 additional clinical MRSA strains was assessed after 1, 2, and 3 h by the direct-transfer-on-plate method.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The MRSA strains used in this study were *S. aureus* ATCC 43300, from the American Type Culture Collection, as well as 10 additional clinical MRSA strains from our collection. Bacteria were stored at −80°C in cryovial bead preservation systems (Microbank; Pro-Laboratory Diagnostics, Richmond Hill, Canada) and streaked onto brain heart infusion (BHI) agar plates before every experiment. Cultures for the preparation of bacterial inocula were grown overnight in BHI broth (Oxoid, Basingstoke, United Kingdom), from a single colony picked on a BHI agar plate, and incubated at 37°C for 16 h. Cultures were subsequently washed 3 times in 0.9% NaCl and serially diluted to achieve the required concentrations used for the inoculation of the unsputtered or Cu-sputtered polyester samples.

Cu deposition onto polyester samples by sputtering and choice of sputtering parameters. Sputtering onto polyester was achieved by direct-current magnetron sputtering (DCMS) or direct-current pulsed magnetron sputtering (DCPMS). Briefly, the positive argon (Ar) ions were accelerated toward a Cu target (obtained from Lesker AG, Hastings, East Sussex, United Kingdom) by applying a voltage of −400 V. The bombarding of the target dislodged Cu atoms that were then deposited onto the polyester substrate, forming Cu-sputtered polyester. The thickness of the Cu layer depends on the duration of the sputtering: the longer the time, the thicker the Cu layer. In our experimental setting, the plasma vacuum had a pressure of 0.1 Pa, and the distance between the Cu target and the polyester was ~10 cm. The deposition current was 300 mA at −400 V.

Electron microscopy of the Cu samples required the samples to be embedded in epoxy resin and then cross-sectioned with an ultramicrotome up to a thin section of 70 nm. A Philips (Philips Electronics N.V., Netherlands) high-resolution transmission electron microscopy (HR-TEM) CM 300 microscope (field emission gun, 300 kV, and 0.17-nm resolution) and a Philips EM 430 instrument (300 kV, LaB_6, and 0.23-nm resolution) were used to measure the particle sizes of Cu clusters.

Inoculation of unsputtered or Cu-sputtered polyester fabrics. Standardized square fabrics (4 cm^2) of unsputtered (negative control) or Cu-sputtered polyester were used. The samples were sterilized by autoclaving (121°C for 20 min) prior to experimental determinations, handled with sterile plastic tweezers, and used only once for each experiment. Fabrics were loaded with various MRSA inocula (ranging from 10^7 to 10^2 CFU, depending on the experiments) in 20 μl of 0.9% NaCl. Inocula were controlled by viable counts performed by plating 10-fold serial dilutions onto BHI agar plates and counting colonies after 24 h of incubation at 37°C. Typically, a 10^6-CFU inoculum was 10^6 CFU ± 0.3 × 10^6 CFU. Inoculated fabrics were then incubated for 1, 2, or 3 h in a humidified chamber at room temperature. After incubation, MRSA viability was assessed by the four methods described below.

Mechanical detachment. Unsputtered polyester fabrics were inoculated with 10^6 CFU of MRSA and incubated for 2 h. After incubation, polyester fabrics were placed into a 2-ml Eppendorf tube containing 1 ml of 0.9% NaCl, and bacteria were detached by three different methods: (i) vortexing (3 min at 3,200 rpm), (ii) vortexing-sonication-vortexing (30 s at 3,200 rpm, 1 min at 100%, and 30 s at 3,200 rpm, respectively), or (iii) sonication (3 min at 100%) (BactoSonic 14; Bandelin Electronic GmbH, Germany). Durations of vortexing and/or sonication were established by previous work in our laboratory to optimize the detachment efficiency while conserving MRSA viability (A. Trampuz et al., unpublished data). Viable counts were then performed by plating serial dilutions of the resuspended bacteria onto BHI agar plates and counting the colonies after 24 h of incubation at 37°C. For each experiment, three different polyester coupons were inoculated and processed independently.

Microcalorimetry. Replicating bacteria release heat that can be measured by microcalorimetry (23). A microcalorimeter (TAM III; TA Instruments) equipped with 48 channels was used in the isothermal mode (set temperature of 37°C). The heat generated by the samples was continuously monitored over time. Heat flow curves represented the growth of the bacteria. The experiments showed an initial lag phase followed by exponential growth until a peak was reached and a decrease of heat was observed. These phases corresponded to the start of growth, exponential growth, and the stationary phase, respectively. The highest value of the heat flow curve was defined as the peak heat (PH) (in μW), and the time from the start of measurements until peak heat was reached was defined as the time to peak heat (TPH) (in hours).

In order to correlate the PH and TPH with the initial MRSA concentration deposited onto the squares, unsputtered polyester fabrics were inoculated with 10-fold serial dilutions of MRSA (10^7 CFU to 10^2 CFU). After 2 h of incubation, inoculated polyester fabrics were inserted into 4-ml microcalorimeter glass ampoules containing 3 ml of BHI broth. Ampoules were hermetically sealed and inserted into the microcalorimeter, and MRSA growth was monitored as heat production over time up to 72 h. Heat flow curves were analyzed by using TAM Assistant software (v1.1.10.5; TA Instruments). The shape of the heat flow curves as well as PH and TPH parameters for different MRSA concentrations were used to establish a microcalorimetry scale.

Assessments of MRSA viability on Cu-sputtered polyester were then performed. An MRSA inoculum of 10^6 CFU was deposited onto Cu-sputtered polyesters or unsputtered polyesters as controls. Inoculated fabrics were incubated during 1, 2, and 3 h and inserted into microcalorimeter ampoules, as described above. Interpretation of the results was carried out by a comparison of the results with the values obtained for the microcalorimetry scale. All microcalorimetry experiments were performed in triplicate.

Direct transfer onto plates. For the enumeration of microorganisms in the environment, special agar plates can be applied directly onto the tested surface, and the number of colonies can be counted after a period of incubation. Using the same principle, we applied the inoculated polyester directly onto BHI agar plates to transfer the potential viable MRSA colonies from the fabric to the plate.

In order to correlate the number of bacteria transferred from the polyester fabrics onto the agar plate to the bacterial load on the samples, unsputtered polyester fabrics were inoculated with 10-fold serial dilutions of MRSA (10^7 to 10^2 CFU). After 2 h of incubation, inoculated polyester fabrics were deposited onto BHI agar plates, with the bacteria facing the agar. BHI agar plates were separated in four quadrants, and pressure (0.07 N/cm^2) was applied sequentially onto the polyester fabric sample for 1 min in each quadrant by using a 50-ml conical Falcon tube (BD Biosciences, San Jose, CA) filled with 50 ml of water as weight and applied onto the fabric with the cap facing downwards. Of note, an increasing duration of pressure (up to 10 min) did not improve the transfer efficacy (data not shown). Plates were then incubated for 16 h at 37°C before the transferred colonies were evaluated. Transferred colonies were counted in each quadrant, and the sum of the 4 quadrants was determined as the number of transferred colonies (NTC). The NTC obtained for different MRSA concentrations was used to establish a transfer-on-plate scale.

An assessment of MRSA viability on Cu-sputtered polyester fabric was then performed. An MRSA inoculum of 10^6 CFU was deposited onto Cu-sputtered polyester fabrics, along with unsputtered polyester fabrics as controls. Inoculated fabrics were incubated during 1, 2, and 3 h, and transfer onto a plate was carried out as described above for the scale. The reported results were obtained by comparisons of the results with the values obtained for the transfer-on-plate scale. For each experiment, three different polyester samples were inoculated and processed independently.

Stereomicroscopy. Unsputtered and Cu-sputtered polyester samples were inoculated with 10^6 CFU of MRSA and incubated for 2 h in a hu-

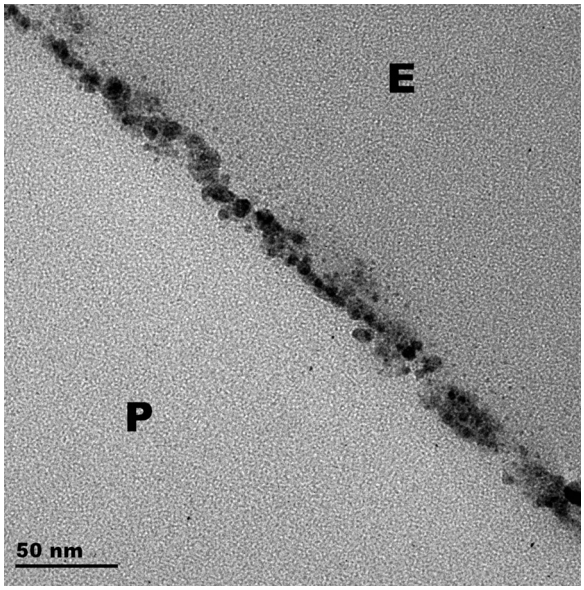

FIG 1 HRTEM of Cu particles sputtered at 300 mA for 160 s onto polyester fabrics. E stands for epoxide, which was used to enrobe the Cu-polyester, and P stands for polyester.

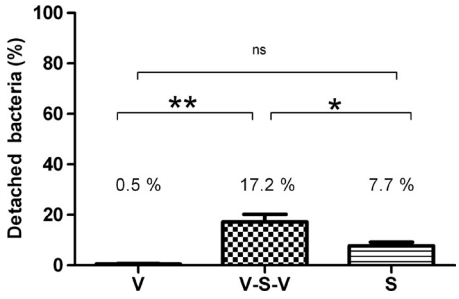

FIG 2 MRSA viability assessed by mechanical detachment. Unsputtered polyester samples were inoculated with 10^6 CFU of MRSA for 2 h, and the detachment of bacteria was performed by either vortexing for 3 min (V); vortexing-sonication-vortexing for 30 s, 1 min, and 30 s, respectively (V-S-V); or sonication for 3 min (S). The percentage of detached bacteria is expressed as the ratio between the MRSA load retrieved after detachment and the MRSA load inoculated onto the fabric. Results are presented as means and standard deviations. One-way ANOVA followed by Tukey's multiple test indicated that means were significantly different (*, $P < 0.05$). ns, not significant.

midified chamber at room temperature. Inoculated polyester fabrics were then stained by using a Filmtracer Live/Dead biofilm viability kit (Molecular Probes, Invitrogen). This kit contains a combination of SYTO9 and propidium iodide fluorochromes for the staining of live and dead cells, respectively. Since polyester fabrics were not transparent, samples could not be observed by confocal microscopy and were observed by fluorescence stereomicroscopy (Leica MZ16 FA; Leica Microsystems GmbH, Wetzlar, Germany), which allowed the illumination and observation of the sample from above. Images were processed by using software provided by the manufacturer (LAS v.1.7.0, build 1240; Leica Microsystems CMS GmbH).

RESULTS

Cu deposition onto polyester samples by sputtering and choice of sputtering parameters. HRTEM results for polyester samples showed most of the Cu particles presenting a diameter of 8 to 15 nm (Fig. 1). Deposition times of 90, 120, and 160 s led to Cu layers of 12, 15, and 20 nm, respectively, as detected by profilometry.

In preliminary experiments, screening of the bactericidal activities of different types of Cu-sputtered polyester fabrics was performed. Samples from two different sputtering types (DCMS and DCPMS) with three different sputtering times (90, 120, and 160 s) were tested. The fabrics were inoculated with 10^6 CFU of MRSA and incubated for 1, 2, and 3 h, and the viability of MRSA was assessed by using the direct-transfer-on-plate method. Samples sputtered for 160 s by using the DCMS technique exhibited the best bactericidal activity, with at least a 4-$\log_{10}$ reduction of the initial inoculum in 1 h (data not shown). Hence, the DCMS sputtering of Cu for 160 s led to the optimal ratio of Cu-loading/cluster size interactions for MRSA inactivation on the sample surface, and these DC-sputtered samples were chosen for all further experiments.

Assessment of bacterial viability by mechanical detachment. Bacteria from samples inoculated with 10^6 CFU of MRSA were detached by vortexing, vortexing-sonication-vortexing, or sonication, and the viability of the resuspended MRSA cells was assessed by viable counts. The detachment efficacy was determined by comparing the number of detached MRSA cells with the number of inoculated MRSA cells, giving a detachment percentage. Detachment percentages of the different methods were then compared. Means and standard deviations of detached percentages of MRSA were 0.5% ± 0.2% by vortexing, 17.2% ± 5.2% by vortexing-sonication-vortexing, and 7.7% ± 2.6% by sonication (Fig. 2). Statistical analysis (one-way analysis of variance [ANOVA] followed by Tukey's multiple test) performed on the viable counts obtained with the three methods indicated that the vortexing-sonication-vortexing method was significantly better than the other two methods. Of note, the slightly increased recovery rate observed for methods which used sonication might be due to the enhanced breaking up of *S. aureus* aggregates, resulting in a higher count of separate colonies on culture plates. However, a recovery rate of only 17% was considered not to be representative of the MRSA load on the samples. In consequence, the mechanical detachment methods were deemed not suitable for the assessment of bacterial viability on the polyester fabrics and were not further investigated with MRSA inoculated onto Cu-sputtered polyesters.

Assessment of bacterial viability by microcalorimetry. Heat flow curves were obtained for 10-fold serial dilutions of MRSA (10^7 to 10^2 CFU) inoculated onto unsputtered polyester and incubated for 2 h. These curves were similar in shape as well as in the height of the PH (~600 μW), and the TPH increased proportionally with the decrease of the MRSA load (Fig. 3A and Table 1). For instance, with the highest inoculum (10^7 CFU), the TPH was only 4.5 h, whereas it was 10.9 h for the lowest inoculum (10^2 CFU). A significant correlation between the inoculated MRSA concentration and the TPH was observed ($r^2 = 0.99$). Therefore, measurements of the TPH could be used to estimate the MRSA load on the polyester fabric.

Heat flow curves obtained with unsputtered and Cu-sputtered polyester fabrics inoculated with 10^6 CFU of MRSA and incubated for 1, 2, or 3 h were compared. Heat flow curves of unsputtered polyester fabrics had similar shapes and values for PH and TPH for different incubation times (Fig. 3B and Table 2). In comparison with the microcalorimetry scale, values for the unsputtered polyester confirmed an initial bacterial load of 10^6 CFU of MRSA.

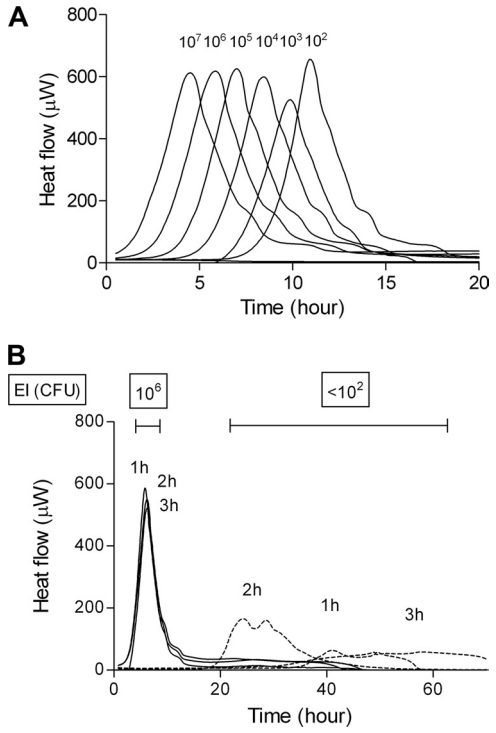

FIG 3 MRSA viability assessed by microcalorimetry. (A) Microcalorimetry scale obtained with heat flow curves of 10-fold dilutions of MRSA (10^7 to 10^2 CFU) deposited onto unsputtered polyester samples and incubated for 2 h. (B) Heat flow curves of 10^6 CFU of MRSA deposited onto unsputtered polyester fabric (solid line) or Cu-sputtered polyester fabric (dashed line) and incubated for 1, 2, or 3 h. The remaining estimated inoculum (EI) (in CFU) of viable MRSA is based on the microcalorimetry scale.

This indicated that MRSA did not lose viability on unsputtered polyester after the different incubation times. In contrast, heat flow curves of Cu-sputtered polyester were less exponential, reached a much lower PH, and exhibited a greatly delayed TPH (Fig. 3B and Table 2). This indicated a remaining bacterial load of MRSA much lower than 10^2 CFU and, hence, at least a 4-$\log_{10}$ reduction from the initial bacterial load within 1 h.

Assessment of bacterial viability by direct transfer onto plates. Transfer onto plates was performed for 10-fold serial dilutions of MRSA (10^7 to 10^0 CFU) inoculated onto unsputtered polyester fabrics and incubated for 2 h. The plates showed that the number of transferred colonies (NTC) correlated with the initial load of MRSA (Fig. 4A). Between 10^4 CFU and 10^2 CFU, the transferred colonies could be counted, and NTC values were reproducible. With an inoculum higher than 10^4 CFU, a confluent bacterial lawn was observed, and the NTC was too high to count. When the inoculum was lower than 10^2 CFU, no transferred colonies were observed, indicating that 10^2 CFU represented the limit of detection for this method. We therefore obtained a semiquantitative scale that allowed estimations of the inoculum size based on the NTC (data not shown).

Direct transfers obtained with unsputtered and Cu-sputtered polyester fabrics inoculated with 10^6 CFU of MRSA and incubated for 1, 2, or 3 h were compared. The transfer of unsputtered polyesters showed the presence of a confluent bacterial lawn after 1, 2, or 3 h (Fig. 4B). Compared with the semiquantitative scale, this indicated a load of about 10^6 CFU of MRSA, showing that no reduction in the bacterial load on unsputtered polyesters occurred after any of the tested incubation times. In contrast, only a few colonies were recovered after the transfer of the Cu-sputtered polyester fabrics onto agar plates (Fig. 4B). Compared with the semiquantitative scale, the amount of transferred colonies corresponded to a remaining bacterial load close to the detection limit, that is, 10^2 CFU of MRSA. This indicated a diminution of almost 4 $\log_{10}$ in the initial bacterial load for any of the tested incubation times. Hence, 1 h of incubation was sufficient to kill 99.99% of the bacteria deposited onto the Cu-sputtered polyester.

A control experiment showed that no bacteriostatic or bactericidal amount of Cu was deposited onto the agar from Cu-sputtered fabrics during the transfer-on-plate procedure. Sterile unsputtered and Cu-sputtered fabrics (without bacteria) were applied onto BHI agar plates as described above. The various MRSA inocula (10^7 to 10^0 CFU) were then deposited onto these areas, and the plates were incubated for 16 h at 37°C. No difference in growth was observed in areas where either unsputtered or Cu-sputtered polyester had been applied.

Assessment of bacterial viability by stereomicroscopy. The spatial distribution and viability of MRSA were assessed by fluorescence stereomicroscopy after 2 h of incubation. Stereomicroscopy images obtained with unsputtered and Cu-sputtered polyester fabrics inoculated with 10^6 CFU of MRSA and incubated for 2 h were compared. Bacteria were observed deep in the mesh of the polyester fabric. After staining, bacteria deposited onto unsputtered polyester fluoresced green, indicating living bacteria (Fig. 5A). In contrast, bacteria on Cu-sputtered polyester fluoresced mostly red, indicating dead bacteria (Fig. 5B). Overall, these observations provided further evidence of the highly bactericidal effect of Cu-sputtered fabrics.

TABLE 1 Assessment of bacterial viability by microcalorimetry on unsputtered polyester fabrics[a]

Initial inoculum (CFU)	Incubation time (h)	TPH for unsputtered polyester (h)	PH for unsputtered polyester (μW)
10^7	2	4.5	613
10^6	2	5.8	618
10^5	2	7	626
10^4	2	8.4	599
10^3	2	9.9	526
10^2	2	10.9	656

[a] Shown are data for the peak heat flow (PH) and time to reach PH (TPH) of unsputtered polyester fabrics incubated for 2 h with 10-fold serial dilutions (10^7 to 10^2 CFU) of MRSA strain ATCC 43300.

TABLE 2 Assessment of bacterial viability by microcalorimetry on unsputtered and Cu-sputtered polyester fabrics[a]

		Unsputtered polyester			Cu-sputtered polyester		
Incubation time (h)	Initial inoculum (CFU)	TPH (h)	PH (μW)	Estimated inoculum (CFU)	TPH (h)	PH (μW)	Estimated inoculum (CFU)
1	10^6	5.8	587	10^6	49.5	58	$<10^2$
2	10^6	6.2	550	10^6	24.3	165	$<10^2$
3	10^6	6.3	522	10^6	40.9	64	$<10^2$

[a] Shown are data for the PH and TPH of unsputtered and Cu-sputtered polyester fabrics incubated with an initial inoculum of 10^6 CFU for 1, 2, or 3 h. The remaining estimated inoculum of viable MRSA is based on the results shown in Table 1.

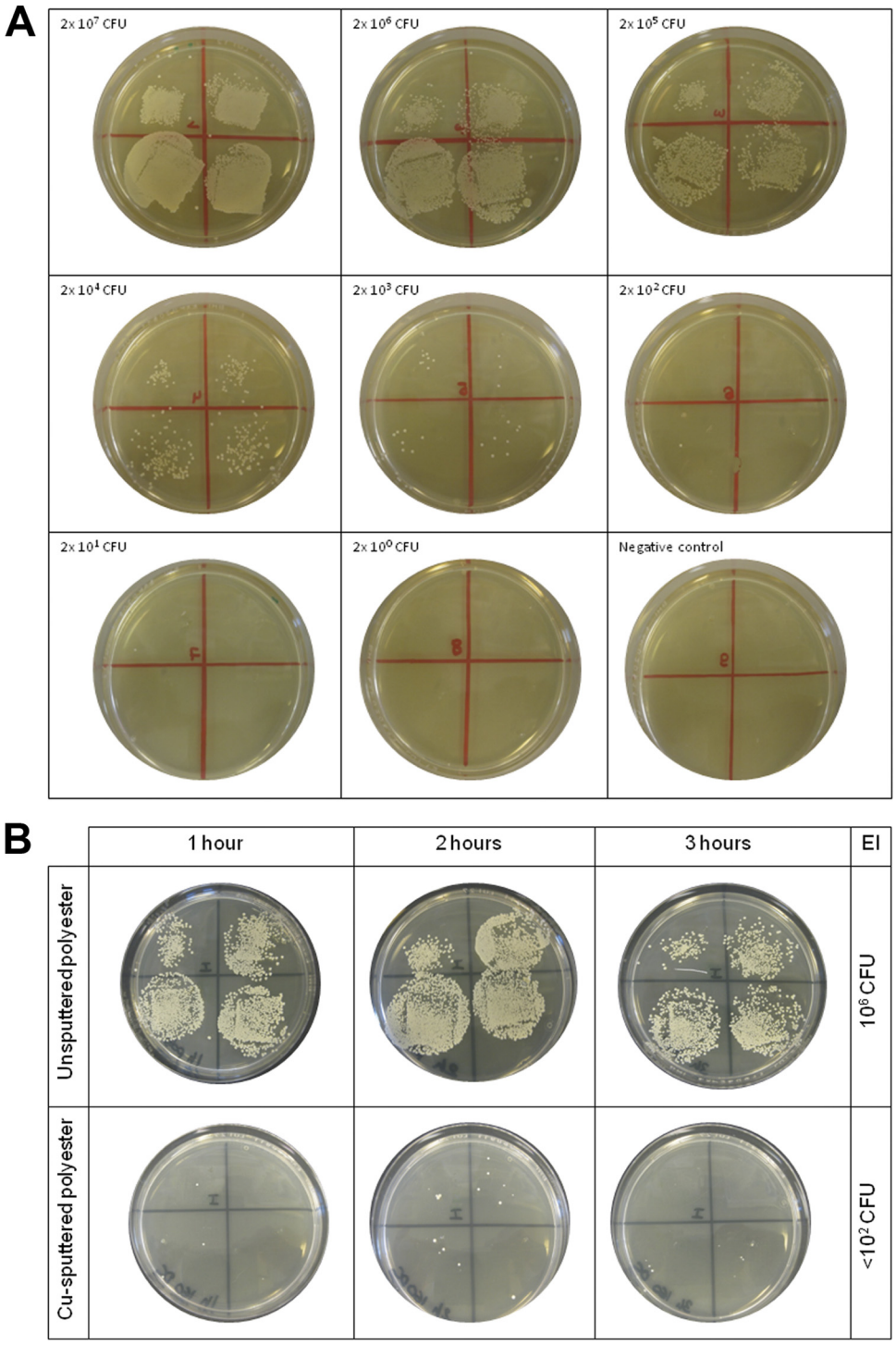

FIG 4 MRSA viability assessed by direct transfer onto plates. (A) Pictures of the transfer onto BHI agar plates of 10-fold dilutions of MRSA (10^7 to 10^2 CFU) deposited onto unsputtered polyester fabrics and incubated for 2 h. (B) Pictures of the transfer onto BHI agar plates of 10^6 CFU of MRSA deposited onto either unsputtered polyester or Cu-sputtered polyester fabrics and incubated for 1, 2, or 3 h. EI, remaining estimated inoculum.

Bactericidal activity of Cu-sputtered polyester against clinical MRSA isolates. The bactericidal activity of Cu-sputtered polyester against MRSA was further tested on 10 additional clinical MRSA strains by using the direct-transfer-on-plate method with 10^6 CFU as the starting inoculum. As observed for strain ATCC 43300, the number of transferred colonies indicated a >3-$\log_{10}$ decrease of the bacterial load after any of the incubation times on Cu-sputtered polyester samples (Fig. 6). Of note, for any clinical isolate, transfer from unsputtered polyester fabrics showed the presence of a confluent bacterial lawn after 1, 2, or 3 h, indicating no loss of bacterial viability.

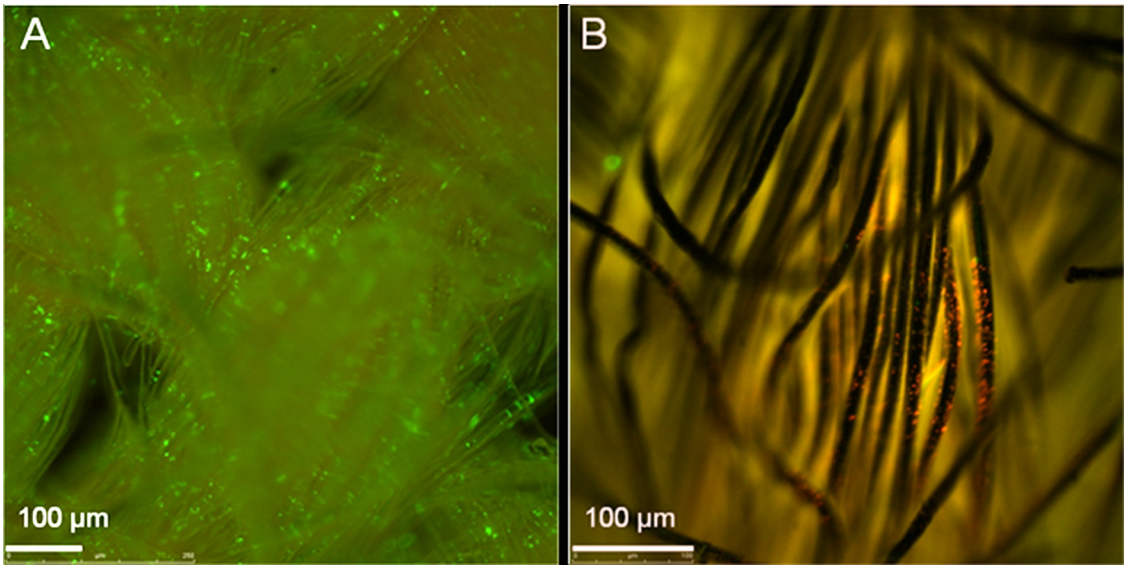

FIG 5 MRSA viability assessed by stereomicroscopy. Shown are fluorescence images of 10^6 CFU of MRSA strain ATCC 43300 deposited onto unsputtered (A) and Cu-sputtered (B) fabrics and stained with a combination of SYTO9 green-propidium iodide after 2 h of incubation.

DISCUSSION

Cu and its alloys have been registered as antimicrobial agents by the U.S. Environmental Protection Agency (EPA), and guidelines have been established to determine the efficacy of Cu alloy surfaces as a sanitizer (8). Typically, a high inoculum of the tested bacteria is spread onto a 1-cm^2 carrier of the metal, and bacterial inactivation is assessed after 120 min of incubation by sonication followed by viable counts. Also, the recovery of bacteria from hard surfaces, such as infected prosthetic materials in humans, can be obtained by mechanical detachment methods such as vortexing and sonication followed by viable counts (5, 13, 22). So far, most studies of Cu surfaces have been conducted with crude Cu, using large amounts of metal and being limited to hard surfaces such as door handles, bed rails, or toilet seats (20, 25). The utility of Cu as an antimicrobial agent for flexible materials such as hospital gowns or bed sheets has received less attention. Covering objects with a thin coating of Cu might be a solution to preserve the Cu properties without the constraints of a hard metal layer and reduce the quantities of the metal used. This stimulates the development of innovative routes to engineer metal-particulate films to reduce or eliminate the prevalence of HCAIs. Sputtering is a method that allows the deposition of a thin layer of metal onto virtually any surface in the form of well-defined atomic layers. In this study, Cu-sputtered fabrics were manufactured by using a DC magnetron that deposited Cu onto polyester, creating a flexible fabric with antimicrobial properties.

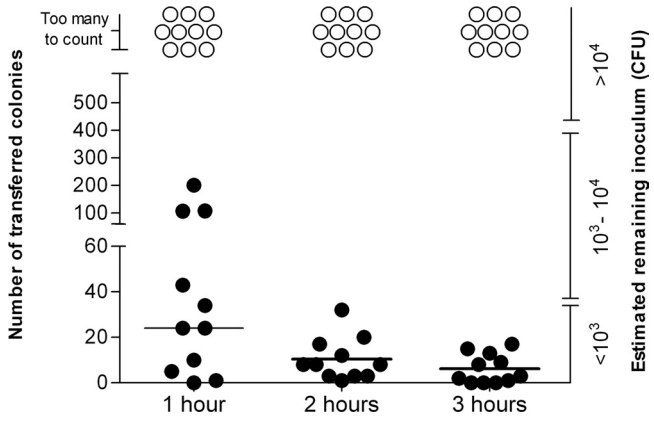

FIG 6 Viability of 10 clinical MRSA isolates on unsputtered or Cu-sputtered polyester fabrics. Each dot represents the number of colonies transferred onto BHI plates (left *x* axis) of an inoculum of 10^6 CFU of 10 clinical MRSA isolates deposited onto unsputtered (white circles) or Cu-sputtered (dark circles) polyester fabrics and incubated for 1, 2, or 3 h. The estimated remaining inoculum of viable MRSA (right *x* axis) is based on the direct-transfer scale (Fig. 4).

We then compared 4 methods to assess the viability of MRSA on unsputtered and Cu-sputtered polyester. We observed that mechanical detachment retrieved, at best, less than one-fifth of the initial amount of MRSA deposited onto the unsputtered polyester. The low efficiency of mechanical detachment methods was probably due to the particular nature of the polyester surface, a tridimensional mesh (ca. 130 μm thick) into which bacteria diffuse and are more difficult to detach than from plain hard surfaces for which sonication has demonstrated a very good detachment efficiency.

Microcalorimetry is an accurate tool for the detection of bacterial growth (3, 23). In the present study, we showed that Cu-sputtered polyesters exhibited an important delay in the time to peak detection, indicating that fewer than 10^2 CFU of MRSA survived after 1 h on the fabric. However, because MRSA cells remained in contact with the Cu-sputtered polyester during the measurement of heat production in the microcalorimeter, this slightly overestimates the bactericidal effect.

Direct transfer onto agar plates combined two advantages. First, this was the method that required the fewest steps between the incubation of MRSA on fabrics and the estimation of viability. Second, it was the most affordable method of all, requiring only agar plates. Because of this, it was determined to be the most simple and reliable method to evaluate the bactericidal activity of Cu-sputtered fabrics.

Fluorescence stereomicroscopy showed that bacteria entered deeply into the fabric and were in direct contact with the polyester fibers. They could be differentiated into live or dead bacteria by using a simple fluorochrome-based staining method.

In conclusion, the combination of direct transfer onto agar plates and stereomicroscopy offered sufficient information to evaluate the bactericidal activity of Cu-sputtered polyester. Our results showed that polyester sputtered with Cu for 160 s exhibited a very high bactericidal efficiency of at least 3 to 4 $\log_{10}$ units against the 11 MRSA strains with 1 h of incubation. Further experiments are required to assess the activity of Cu-sputtered textiles on additional bacterial pathogens which cause HCAIs, including Gram-positive (e.g., vancomycin-resistant enterococci and *C. difficile*) and Gram-negative (e.g., *P. aeruginosa*) bacteria as well as fungi or viruses. When available, Cu-sputtered fabrics might be used to manufacture hospital gowns or bed sheets in order to lower the bacterial load in the environment of the patient and reduce pathogen transmission between health care workers and patients or between patients. Manufacturing costs as well as the resistance of such fabrics to laundering require additional studies, but Cu-sputtered textiles show promise as materials to reduce the transmission of health care-associated pathogens.

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