

## Article

# Weathering and Antimicrobial Properties of Laminate and Powder Coatings Containing Silver Phosphate Glass Used as High-Touch Surfaces

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**Abstract:** Increasing the use of hygienic high-touch surfaces with antimicrobial properties in health care and public spaces is one way to hinder the spread of bacteria and infections. This study investigates the antimicrobial efficacy and surface reactivity of commercial laminate and powder coated surfaces treated with silver-doped phosphate glass as antimicrobial additive towards two model bacterial strains, *Escherichia coli* and *Bacillus subtilis*, in relation to surface weathering and repeated cleaning. High-touch conditions in indoor environments were simulated by different extents of pre-weathering (repeated daily cycles in relative humidity at constant temperature) and simplified fingerprint contact by depositing small droplets of artificial sweat. The results elucidate that the antimicrobial efficacy was highly bacteria dependent (Gram-positive or Gram-negative), not hampered by differences in surface weathering but influenced by the amount of silver-doped additive. No detectable amounts of silver were observed at the top surfaces, though silver was released into artificial sweat in concentrations a thousand times lower than regulatory threshold values stipulated for materials and polymers in food contact. Surface cleaning with an oxidizing chemical agent was more efficient in killing bacteria compared with an agent composed of biologically degradable constituents. Cleaning with the oxidizing agent resulted further in increased wettability and presence of residues on the surfaces, effects that were beneficial from an antimicrobial efficacy perspective.

**Keywords:** antimicrobial surfaces; silver; indoor hygiene; laminate; powder coating

## 1. Introduction

Pandemics, antimicrobial resistance (AMR), and healthcare-associated infections (HAIs) are examples of complex societal challenges that require collaborative interdisciplinary efforts of stakeholders spanning multiple sectors and academic disciplines [1–3]. Solutions to such complex problems are often referred to as multi-sector innovations [1,4].

During the last two years, intensive attention has been paid to improved hand hygiene and cleanliness of indoor spaces to prevent the spread of the COVID-19 pandemic. Less active measures in public communication have been taken related to antimicrobial-resistant

bacteria causing HAIs, which by 2050 are estimated to kill more people than cancer, reaching up to 10 million deaths per year [5]. Antimicrobial-resistant bacteria pose a threat not only in hospitals causing HAIs but are also detected in public spaces where many people spend their time or pass through [6]. Thus, the prevention of infection transmission is getting more attention, especially in public spaces. Usually, measures against more severe infections like COVID-19 and AMR are often also effective against traditional causes of infections, such as influenza, common flu, and stomach flu. The economic consequences of infections transmitted mostly indoors can be extremely high. For example, the costs of the COVID-19 pandemic have been estimated to be over \$16 trillion in the USA alone [7].

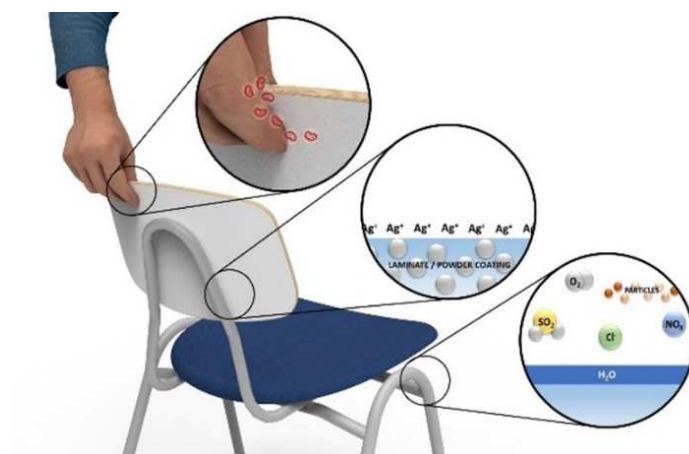
Research on different technologies aiming to improve infection prevention and control has been carried out for decades. Technologies based on, for example, anti-microbial coatings on touch surfaces aiming to improve the level of hygiene and reduce the transmission of infections in building environments are already commercially available. However, individual products are not enough to break the infection chain in indoor environments. Pioneering work in consolidating all central indoor hygiene elements (air, water, surfaces) to a comprehensive indoor hygiene concept (IHC) has been done by Satakunta University of Applied Sciences (SAMK). Several related studies have been conducted on IHC effects on the microbial burden in public spaces such as kindergartens, hospitals, and elderly care homes [8–10]. The main idea of IHC is to create an innovative and comprehensive overarching solution implemented already during the construction phase in order to improve the level of hygiene during the whole life cycle of the building. To provide information on risk evaluation, prudent use, and cleaning of antimicrobial coatings, SAMK has actively participated in the European COST Action network AMiCI (Antimicrobial coating innovations to prevent infectious diseases) [11–15].

The HygTech alliance [16], a group of Finnish companies, has collaborated with SAMK since 2013 to create comprehensive solutions for indoor surfaces in shared public spaces including antimicrobial coatings for waiting areas, bathrooms, and clinical examination rooms. Industry–academia collaborations are hence important when creating innovative solutions, during operation in health care settings, and when proving and elucidating effects of new technologies. It is further crucial that all parties in a construction project of, e.g., a new hospital, from architects, electrical, and HVAC (heating, ventilation, and air conditioning) engineers to maintenance and cleaning personnel understand the importance of the concept.

A way forward to improve the level of indoor hygiene by minimizing infection transmission is to use materials that have inherent antimicrobial properties (e.g., silver-containing materials and copper-based alloys) or that are coated or treated with antimicrobial additives for high-touch applications [12,17]. This can be accomplished by using materials with antimicrobial properties on critical, frequently touched surfaces, such as door handles, handrails, chair armrests, and toilet flush buttons, combined with traditional infection prevention and control procedures (including proper hand hygiene, efficient cleaning, and disinfection) and prudent use of antibiotics [18]. However, there are conflicting views on the safety of using antimicrobial materials and coatings from an AMR perspective [14]. There is hence a pronounced need to elucidate the benefits and value of the indoor hygiene concept [12,19,20]. The antimicrobial efficiency of silver metal has been known for thousands of years, connected to a slow release of silver ions when used in a variety of applications both for consumers and in the medical sector, e.g., [21]. Silver ions have shown a high antimicrobial capacity in water already at very low concentrations [22]. Silver-doped phosphate glass is an example of an antimicrobial additive from which the release of silver from the soluble glass structure can be controlled [23,24]. The antimicrobial properties of silver-doped phosphate glass in commercially available products have been reported for, e.g., coatings on doors and door handles, in wooden furniture, textiles, and electrical switches in health care settings [25–28].

The aim of this paper is to elucidate the influence of simulated indoor weathering (repeated dry/wet daily cycles at constant temperature) and simulated fingerprint contact

on the antimicrobial properties of commercial laminate and powder coatings treated with silver-doped phosphate glass as an antimicrobial additive (Figure 1). Three research questions were addressed: (i) is the antimicrobial efficacy of silver-treated laminate surfaces and powder coatings (IH materials) reduced upon simulated surface weathering at atmospheric indoor conditions and simulated fingerprint contact? (with and without deposition of artificial sweat), (ii) is the silver content within the laminates or powder coatings present in the outermost top surface, and are released concentrations of silver measurable? and (iii) can repeated manual surface cleaning with commercial cleaning agents of different chemistry hinder bacterial growth on silver-treated laminate surfaces and will repeated cleaning influence their surface properties and antimicrobial efficiency?



**Figure 1.** Schematic illustration of indoor weathering and bacteria transfer via fingerprint contact with antimicrobial high-touch surfaces.

Weathering and antimicrobial studies were conducted following a newly elaborated test methodology [29] proven able to simulate indoor weathering by exposing laminate and powder-coated samples to daily cycles of dry and wet conditions in a climatic chamber (thin aqueous layer conditions [19]) and simulating fingerprint contact by repeatedly spraying small drops of artificial sweat onto the surfaces. The differently weathered surfaces (1 day up to 4 weeks) were exposed to model bacterial strains (*Escherichia coli* and *Bacillus subtilis*) added to the surfaces in small droplets mimicking fingerprint bacteria transfer [29]. Gram-positive and Gram-negative model bacteria were used to mitigate any risk of transfer and spread of pathogenic bacteria during the characterization. Unexposed and exposed surfaces were evaluated in terms of their antimicrobial efficiency (viability), outermost surface composition (X-ray photoelectron spectroscopy), extent of released silver (atomic absorption spectroscopy), and changes in surface appearance (scanning electron microscopy) and characteristics (wettability).

## 2. Materials and Methods

### 2.1. Chemicals and Solutions

Fingerprint contact was simulated using artificial sweat (ASW) prepared according to the EN 1811 standard [30], mixing 5.0 g/L sodium chloride (NaCl), 1.0 g/L urea ( $\text{CH}_4\text{N}_2\text{O}$ ), 1.0 g/L lactic acid ( $\text{C}_3\text{H}_6\text{O}_3$ ), and ultrapure water (Milli-Q, 18.2 M $\Omega$ ·cm, Millipore, Solna, Sweden) to a pH of  $6.5 \pm 0.05$  (adjusted by adding NaOH). ASW was freshly prepared and used within 8 h of preparation to be pre-deposited on the high-touch surfaces in a well-controlled way by using an airbrush (Aztek-A220 Broad Stroke Airbrush system, Aztek, Cleveland, OH, USA). All chemicals (analytical grades) were obtained from VWR, Stockholm, Sweden.

Silver-doped phosphate glass (non-crystalline, particle size  $<5 \mu\text{m}$ , CAS no: 308069-39-8), used as an antimicrobial additive in the laminate and powder coatings, was obtained from BioCote<sup>®</sup>, Coventry, UK (Product name: B85003). The product consists of sparingly soluble

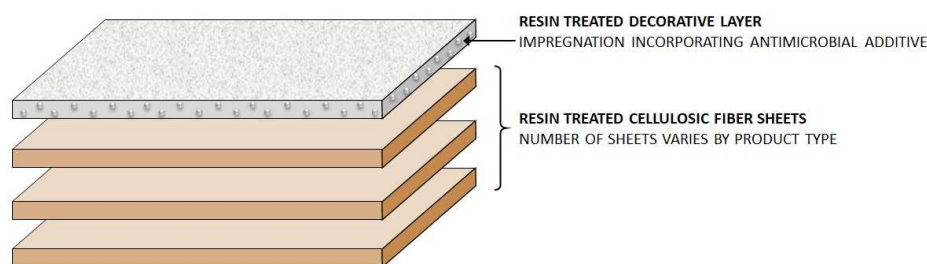
silver ions incorporated into a glass matrix, which provides a slow release of silver ions to the surface of the product. Information on its presence in the laminate and powder coatings is given in Section 2.2. The manufacturer of the antimicrobial additive reports antimicrobial effects on both Gram-positive and Gram-negative bacteria and fungi [31]. Specific surface area measurements of the additive particles using the BET (Brunauer, Emmett and Teller) technique (3Flex, Micromeritics, Norcross, GA, USA) revealed a surface area of  $8.23 \pm 0.187 \text{ m}^2/\text{g}$ .

The effect of repeated cleaning on the antimicrobial efficiency of the laminate surfaces was evaluated using two different commercially available cleaning agents of different chemical characteristics: Plusclean, Kiilto Pro, Finland [32] (bio-based detergent) and Erisan Oxy+, Kiilto Pro, Tampere, Finland [33] (oxidizing detergent). Cleaning was conducted using concentrations suggested by the suppliers. The Plusclean detergent solution (non-ionic surfactants < 5%, Soap < 5%, pH  $9.65 \pm 0.05$ ) was diluted in water to 50 g/L, investigating effects of two different (5% and 10%) cleaning agent concentrations. Parallel cleaning studies (2 and 5%) were performed using the Erisan Oxy+ detergent (sodium percarbonate > 30%, TAED (tetraacetyletyldiamin) 5–15%, Complexing agents > 30%, Non-ionic surfactants < 5%; pH of  $7.3 \pm 0.04$ ).

## 2.2. Commercial Surface

### 2.2.1. Laminate

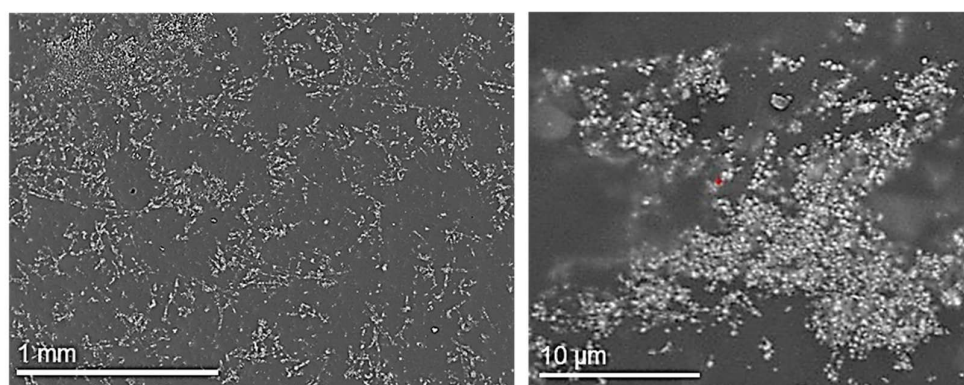
Decorative high-pressure laminate (HPL) surfaces treated with antibacterial silver-doped phosphate glass (BioCote<sup>®</sup>) were provided by ISKU, Lahti, Finland, Figure 2. This multilayered commercial product is manufactured at high temperature ( $\geq 120 \text{ }^\circ\text{C}$ ) and high specific pressure ( $\geq 5 \text{ MPa}$ ). Following the CSN EN 438 standard [34] definition, the material consists of layers of cellulosic fibrous materials (paper) impregnated with thermosetting resins, bonded together by means of a high-pressure process. The impregnation of the cellulosic fibers with thermosetting resin enables the fibers to become saturated with the resin prior to the lamination process. This is followed by a series of oven drying and curing steps to achieve the desired degree of polymerization of the resin layers and a homogeneous non-porous material of required density ( $\geq 1.35 \text{ g/cm}^3$ ) and surface finish.



**Figure 2.** Schematic illustration of the laminated surface treated with silver phosphate glass as antimicrobial additive in the top layer.

The addition of the antimicrobial additive to the commercial laminate investigated in this study is only conducted to the resin used for the top-most decorative layer (Figure 2).

White laminate samples ( $5 \times 5 \text{ cm}^2$  and  $1.5 \times 1.5 \text{ cm}^2$ ) for testing were supplied by ISKU. The white surface appearance originates from titanium dioxide ( $\text{TiO}_2$ ) pigment non-evenly distributed within the resin-treated outer layer, seen as areas of white particles in the SEM images of Figure 3.



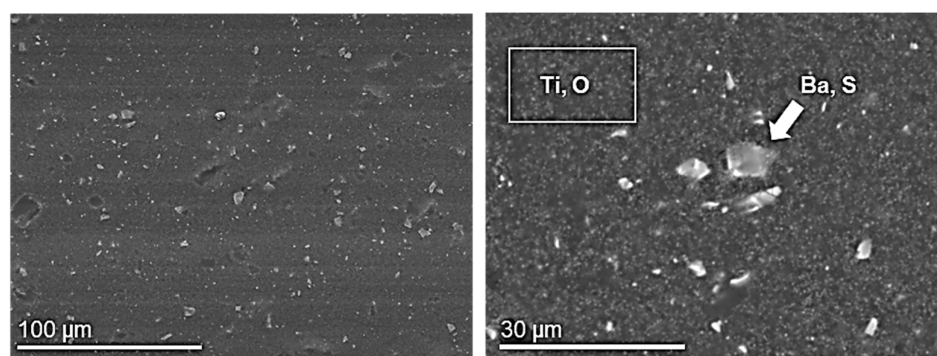
**Figure 3.** Scanning electron microscopy (SEM) images of the as-received laminate surface with areas of  $\text{TiO}_2$  particles providing the whitish surface appearance.

Area analysis by means of energy dispersive spectroscopy (EDS, experimental details given below), providing  $\mu\text{m}$ -depth information of a larger surface ( $\approx 100 \times 100 \mu\text{m}^2$ ), revealed nitrogen, carbon, and oxygen to be the main elements, with small amounts of sodium and titanium (Ti). Similar findings, which also detected silicon and calcium but no titanium, were observed in the outermost 5–10 nm top surface using X-ray photoelectron spectroscopy (XPS, experimental details given below) analyzing  $0.35 \mu\text{m}^2$  sized areas. Neither silver nor phosphorous from the silver-doped phosphate glass additive was observed using either EDS or XPS. This is probably related to its low content within the resin-treated outer layer, estimated from the amount of antimicrobial additive added into the liquid resin. Assuming a uniform distribution of the additive within the resin, its amount would correspond to 0.12 to  $0.18 \text{ g/m}^2$  ( $12\text{--}18 \mu\text{g/cm}^2$ ). With an outer laminate layer thickness of 0.8 mm, this would correspond to a total content of  $150\text{--}225 \mu\text{g additive/cm}^3$ . By further assuming a maximum silver content of 20 wt.% [24] in the silver phosphate glass, the total amount of silver in the outer laminate layer would be less than  $3.6 \mu\text{g/cm}^2$ .

### 2.2.2. Powder Coated Surfaces

The investigated powder coatings were supplied by Teknos Ltd., Rajamäki, Finland, and based on their Infralit™ (Rajamäki, Finland) epoxy-polyester hygienic powder coating series. These coatings are commercially commonly used on surfaces that require high levels of hygiene in hospitals, schools, and meeting rooms, on door handles and hospital beds. The active antimicrobial additive is silver-doped phosphate glass based on the BioCote® technology. Based on supplier information, the coatings show a very good antimicrobial performance following the ISO 22196:2011 standard [35] with >95% and up to 99.99% reduction in viability for both *Escherichia Coli* and Methicillin-resistant *Staphylococcus Aureus* (MRSA) [36].

Two different silver-doped phosphate glass concentrations in the powder coating on aluminum substrates ( $1.5 \times 1.5 \text{ cm}^2$ ) were investigated from a research perspective. The first series with an active amount of 0.5 wt.% (coating A) and a second series with twice the content of the first series (coating B). Assuming a uniform distribution of the additive in the coatings, this amount corresponds to a total amount of 65 and  $129 \mu\text{g additive/cm}^2$  for coating A and B, respectively. Assuming a maximum concentration of 20 wt.% silver in the additive [24], the total amount of silver in the outer surfaces of coating A and B would be less than 13 and  $26 \mu\text{g/cm}^2$ , respectively. No silver could be discerned by means of neither EDS nor XPS. The main elements of the powder coating were, in addition to carbon and oxygen, titanium, barium (Ba), sulfur (S), and minor amounts of strontium (Sr). Barium and sulfur were predominantly present in an atomic ratio of 1:1 in the larger sized particles and unevenly distributed over the surfaces, which implies the presence of  $\text{BaSO}_4$ . Titanium was more homogeneously distributed within the coating, as seen in Figure 4. Both  $\text{BaSO}_4$  and  $\text{TiO}_2$  are constituents of the white pigment used for the investigated powder coating.



**Figure 4.** SEM images of the as-received powder coating B and main elements identified by means of EDS.

### 2.3. Surface Pre-Weathering

The test methodology, recently elaborated to mimic and study the effects of weathering of high-touch surfaces at indoor conditions and simulated fingerprint contact [29] on their surface characteristics and antimicrobial properties at indoor hygiene (IH) conditions, was employed on the laminate and the powder coatings described above.

Pre-weathering was conducted in a climatic chamber (Weiss WK3 340/40, Reiskirchen, Germany) using daily cyclic dry/wet conditions at a constant temperature of 25 °C (90% relative humidity (RH) for 4 h, <10% RH for 2 h, 90% RH for 16 h, and <10% RH for 2 h) for periods up to 4 weeks. The surfaces were during the pre-weathering treatment sprayed with artificial sweat (ASW) once a day and collected after 1 day, 1 week, 2 weeks, and 4 weeks for surface characterization, silver release investigations, and antimicrobial property investigations, as seen below. Parallel exposures were conducted on surfaces without any ASW deposition.

### 2.4. Antimicrobial Testing

Antimicrobial testing was conducted on triplicate samples using Gram-negative and Gram-positive model bacteria, *Escherichia coli* and *Bacillus subtilis*, possible to test in a biosafety level 1 laboratory, thereby assessing differences between the differently weathered surfaces of laminate and powder coatings of different silver additive content and to minimize the risk of transfer of bacteria to the analytical instruments.

Nutrient agar (NA) medium was prepared by mixing 8 g of nutrient broth and 15 g agar in 1 L water autoclaved at a temperature of 121 °C for 15 min using saturated steam and a pressure of 15 psi. The nutrient broth (NB) was prepared without adding agar to the nutrient broth powder and sterilized using an autoclave to prepare bacteria in liquid suspension. The sterilized nutrient agar was poured into sterile Petri dishes, where it was solidified at room temperature and later stored at 4 °C until further use. The ASW solution was sterilized using a 0.2 μm syringe filter (Filtropur S, 0.2, Sarstedt, Germany) and checked by plating 100 μL sterile ASW onto NA and incubated at 37 °C for 24 h. No bacteria growth was discerned.

Bacteria cultures, *Escherichia coli* (*E. coli*, DSM no. 498) and *Bacillus subtilis* (*Bacillus*, DSM no. 10), were grown in nutrient broth (NB) medium overnight (~20 h) at 37 °C and 30 °C with a constant shaking incubator of 190 rpm/min (Infors HT minitron), respectively. The bacteria cultures were then centrifuged (Hermle Z206A centrifuge) at 2500 rpm for 5 min to remove the NB medium, and the bacteria cell pellet was collected and suspended in sterilized ASW. This was followed by centrifugation at 2500 rpm for another 5 min. The supernatant was discarded, and the bacteria cells were once more suspended in ASW. The rationale behind using ASW as the bacteria medium was to mimic the transfer of bacteria during fingerprint contact with high-touch surfaces [29].

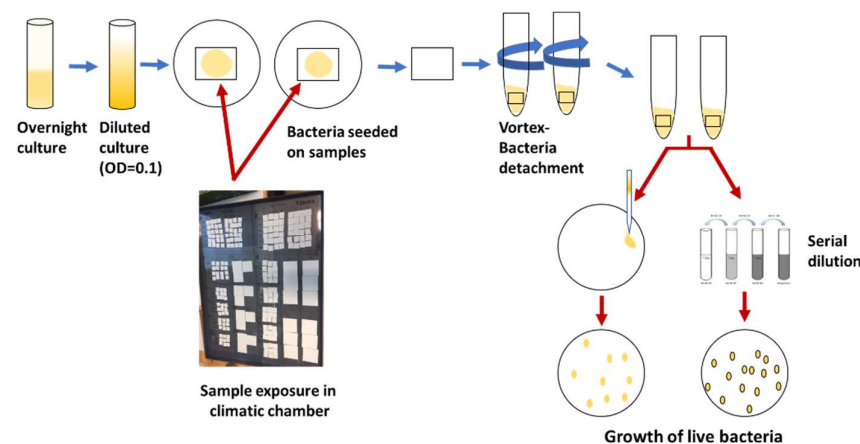
The optical density (OD) of the bacteria cell culture was determined at a wavelength of 600 nm using a Biowave DNA spectrometer (Biochrom Ltd., Cambridge, UK) and diluted

with ASW to  $OD = 0.1$ . The  $OD_{600}$  value was determined by calculating and setting the  $OD$  of artificial sweat without bacteria as zero. The diluted cultures ( $12 \mu\text{L}$ ) were then deposited onto the samples (as described in [29]), incubated in sterile Petri dishes (autoclaved as described above), covered with a lid at room temperature, and removed after 20 min. All workplaces, such as the table for the experiment, were frequently disinfected using concentrated alcohol (70%). A Bunsen burner was used to keep the surrounding air sterile.

After incubation, the samples were placed in sterile plastic tubes (Falcon tubes, Sarstedt, Germany) containing ASW (8 mL) and vortexed for 1 min with high speed (Ika Works Inc., Wilmington, NC, USA) to detach loosely adherent bacteria from the surfaces. A volume of  $100 \mu\text{L}$  of the vortexed solution was plated and spread on a NA plate using an L-shaped glass spreader and incubated at  $37^\circ\text{C}$  (*E. coli*) and  $30^\circ\text{C}$  (*Bacillus*) for 24 h. In parallel, a serial dilution of the vortexed solution ( $100 \mu\text{L}$ ) was prepared and spread onto a NA plate and incubated for 24 h at  $37^\circ\text{C}$  and  $30^\circ\text{C}$ , respectively, to determine the presence of live bacteria detached from the surface.

The growth of live bacteria was observed after 24 h of incubation, counted the number of bacterial colonies and calculated as colony forming units (CFU) by the number of colonies multiplied with the dilution factor. NA plates were used to quantify the viable number of bacteria and/or colonies per mL detached via vortexing from the duplicate surfaces of bacteria-incubated samples of different extent of pre-weathering.

A schematic illustration of the different experimental steps in antimicrobial testing is given in Figure 5. Microscopic glass slides, not pre-weathered but sterilized in an autoclave, were used as control and followed the same procedure described above.



**Figure 5.** Schematic illustration of the experimental steps for antimicrobial testing of laminate and powder coatings treated with silver-doped phosphate glass as antimicrobial agent.

## 2.5. Surface Characterization and Silver Release Studies

### 2.5.1. Surface Appearance and Chemical Composition

SEM (Scanning electron microscopy) analyses were conducted on as-received and selected weathered, cleaned, and unexposed surfaces with and without bacteria with the aim to observe if the weathering process, the cleaning procedure, and or the interactions with the bacteria would influence the top-surface appearance of the laminate and powder coating surfaces. Secondary electron images were acquired by means of an FEI-XL 30 series instrument equipped with an Oxford X-Max SDD (Silicon Drift Detector)  $20 \text{ mm}^2$  EDS system at accelerating voltages of 5 kV and 15 kV for surfaces with and without bacteria, respectively. The surfaces were coated with a thin (40–60 nm) layer of gold to avoid extensive surface charging during the measurements.

XPS (X-ray photoelectron spectroscopy) studies were primarily performed to assess whether silver was possible to observe at the outermost 5–10 nm surface of the laminate and the powder coatings before and after exposure to bacteria as well as after repeated cleaning. The outermost composition of the silver-doped phosphate glass particles only

was also investigated. The measurements were conducted using an UltraDLD spectrometer (Kratos Analytical, Manchester, UK) equipped with an Al x-ray monochromatic source operating at 150 W (10 mA, 15 kV). Wide and high-resolution spectra were acquired on Na 1s, C 1s, O 1s, Ca 2p, P 2p, Si 2p, Al 2p, and Ag 3d. Energy correction was made against the C 1s peak of adventitious carbon at 285.0 eV.

### 2.5.2. Silver Release in Artificial Sweat

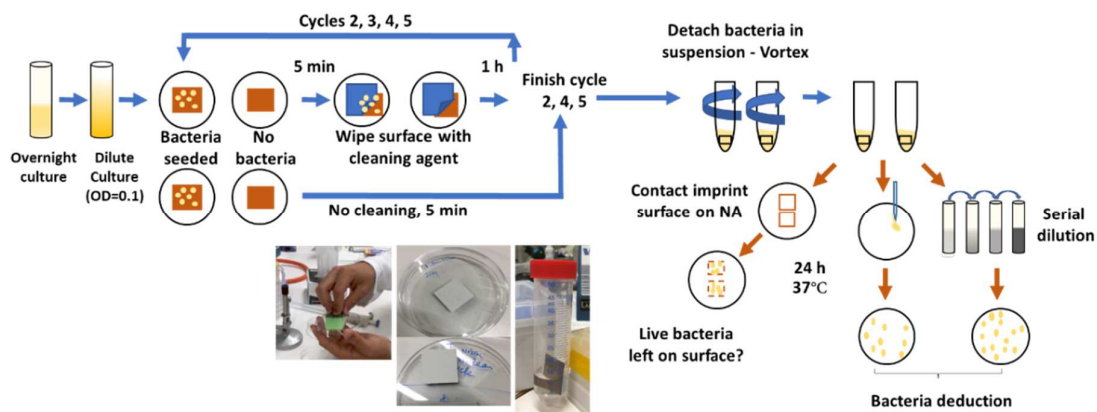
Triplicate samples (2.25 cm<sup>2</sup>) of the weather laminate and powder coated surfaces of different silver-doped phosphate glass content (BioCote<sup>®</sup>) with and without ASW deposition were completely immersed at tilted (45°) conditions in 3.5 mL fresh ASW (i.e., a surface area to solution volume ratio of 0.6 cm<sup>-1</sup>) in 10 mL large exposure vessels (Wheaton<sup>®</sup> sodalime glass with low-density polyethylene (LDPE) snap cap (Mainz, Germany, VWR, Sweden). Immersions were conducted at 30 °C under dark conditions in an incubator for 4, 24 and 168 h following the EN1811 standard [30]. Triplicate samples of two loadings, 5 mg and 50 mg, of the silver phosphate glass powder (BioCote<sup>®</sup>), was immersed in 50 mL of ASW (Nalgene<sup>®</sup> (Rochester, NY, USA) 60 mL polymethylpentene (PMP) vessels with polypropylene (PP) screw caps and exposed at dark conditions for 4 h at 30 °C in an incubator. Blank reference solutions (ASW only) were exposed in parallel to correct for background concentrations of silver for each condition. It should be noted that the immersion conditions in solution as stipulated by the EN1811 standard are different compared to the thin aqueous layer conditions prevailing at indoor conditions [19]. Nevertheless, as no standardized way is available for metal release testing at thin film conditions the test is relevant to assess released amounts of silver and compare with available regulatory threshold values for silver migration, see Section 3.2.

Total concentrations of released silver were analyzed employing Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS) using a PerkinElmer AA800 Analyst instrument operated at standard conditions. Prior to analyses of total amounts of released silver, the test solutions were digested to a pH < 2 using 20 µL 65% HNO<sub>3</sub>. Calibration standards 0, 15, 30 and 60 mg/L were used, and quality control samples were run every 4th sample. Triplicate readings were done for each sample. The limits of detection (LOD) and quantification (LOQ) were 0.2 µg/L and 0.5 µg/L, which correspond to 0.0003 and 0.0008 µg silver/cm<sup>2</sup>, respectively. Calibration standards of 0, 30, 60, and 80 µg/L were used for titanium with LOD and LOQ of 3 and 8 µg/L, respectively. No measurable concentrations of released titanium (<LOD) were observed from the white TiO<sub>2</sub>-treated laminate surfaces.

### 2.6. Cleaning of Bacteria Coated Surfaces and Bacteria Viability Studies

To mimic manual daily cleaning of IH surfaces at, e.g., a school or a hospital, two different commercially available detergents were investigated to assess their effects on the surface characteristics and antimicrobial properties. The cleaning agents, bacterial cell suspensions, and ASW were prepared as described above. Each laminate sample (triplicates) was placed in a sterile Petri dish with a tape stick on one side of the sample surface, followed by the addition of 8 droplets (7.5 µL) of bacteria cell suspensions or ASW (control) on each sample before being incubated at room temperature (RT) for 5 min (Figure 6). After the incubation, the samples were either cleaned or not cleaned with the commercial detergent (3 mL, 2 or 5% concentration). A non-scratch kitchen sponge (similar to Scotch Brite) sized 3.6 × 6 cm<sup>2</sup> was soaked in 3 mL of the detergent and used to clean the surfaces in a specific direction (from left to right side) once or repeated times applying manual pressure (equivalent to a load of 300 g).



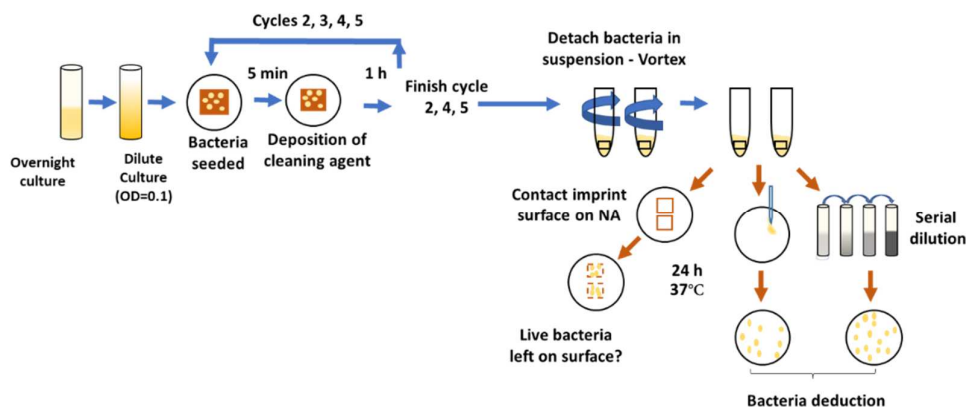


**Figure 6.** Schematic illustration of the experimental steps conducted to investigate the antimicrobial efficiency of repeated cleaning of laminate surfaces.

After cleaning, the samples were left to dry for 1 h at room temperature since some detergent solution remained on the surfaces after the cleaning step, followed by re-deposition of bacteria cell suspension (*E. coli*) or ASW for the second cleaning cycle, incubated at RT for 5 min, followed by 1 h of drying. These steps were repeated following the same protocol until 5 cleaning cycles were completed. Two samples were removed for viability testing after the 2nd, 4th, and 5th cleaning cycles. Non-cleaned samples were removed for viability testing immediately after 5 min of incubation with the bacteria.

Bacteria viability was determined by placing the laminate samples in 20 mL ASW, vortexing for 1 min at high speed to detach adhered bacteria, followed by spreading 100  $\mu$ L of the solution onto NA incubated at 37  $^{\circ}$ C for 24 h (Figure 6). Serial dilution of the vortexed samples was prepared, plated on NA, and incubated at 37  $^{\circ}$ C for 24 h to determine the extent of live bacteria in solution (CFU/mL). After vortexing, the samples were placed onto NA plates to detach the bacteria from the surface (imprinting). Two replicates were investigated for each treatment.

In order to test the antimicrobial efficiency of the detergent only, without any influence of manual mechanical abrasion using the sponge, a bacteria cell solution (7.5  $\mu$ L) was deposited onto the laminate surface and incubated for 5 min, followed by the deposition of detergent solution (7.5  $\mu$ L /sample) on the bacteria deposited spots, and incubated for 1 h, followed by the deposition of another bacteria solution on the same spot for the 2nd cleaning cycle. This procedure was repeated up to 5 cycles, and the number of live bacteria present on the surfaces was determined after the 2nd, 4th, and 5th cycles (Figure 7). The viability study was conducted for two detergent concentrations (Erisan Oxy+; 2 and 5% and Plusclean; 5 and 10%).



**Figure 7.** Schematic illustration of the experimental steps to assess the antimicrobial efficiency of the cleaning agents without any influence of manual abrasion.

The number of live bacteria on the laminate surfaces was determined as described above. To assess whether repeated cleaning would change the surface characteristics in terms of composition and wettability, and thereby the antimicrobial efficiency, repeated wiping (40 times) with the different cleaning agents was conducted. The antimicrobial effect was investigated after 20 min, as described above (c.f. Figure 5). Differences in wettability and surface composition were assessed by means of static water contact angle measurements (Pocket Goniometer PGX, Fibro system, Stockholm, Sweden) and XPS (as described above).

### 3. Results

The following research questions were raised and addressed below:

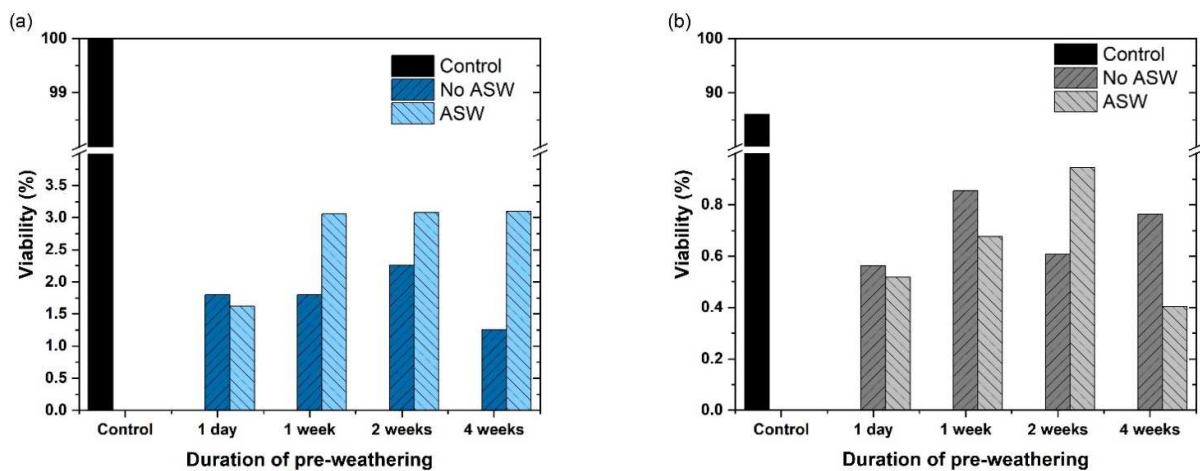
- Is the antimicrobial efficacy of IH materials of laminate and powder coatings treated with a silver-doped phosphate glass additive reduced upon simulated surface weathering and fingerprint contact at atmospheric indoor conditions?
- Is the silver content within the treated materials in measurable amounts in the outermost top surface, and are released concentrations of silver in artificial sweat measurable?
- Can surface cleaning with commercial cleaning agents hinder bacterial growth on the silver-treated laminate surfaces, and will repeated cleaning influence the surface characteristics and their antimicrobial efficiency?

#### 3.1. Changes in Antimicrobial Efficacy on Differently Weathered (No Wear) IH Surfaces

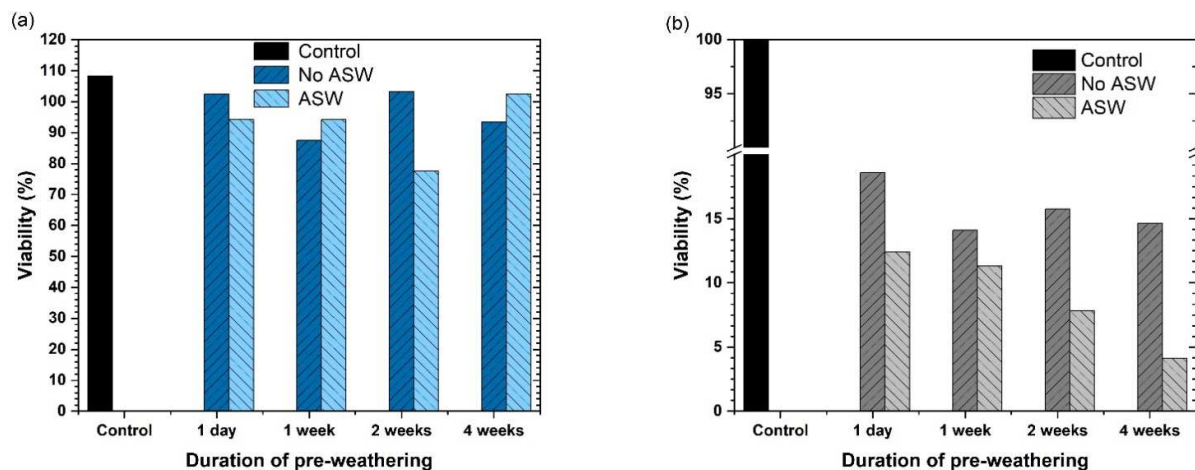
The antimicrobial efficacy of the differently pre-weathered (1 day, 1, 2, and 4 weeks mimicking indoor conditions and fingerprint contact) laminate and powder coated surfaces treated with silver-doped phosphate glass was evaluated using the model Gram-negative bacterial strain *Escherichia coli* and the Gram-positive bacterium *Bacillus subtilis*. Deposition of ASW droplets containing bacteria onto the surfaces was conducted to mimic simplified fingerprint contact. The reduction in viability of *E. coli* bacteria (OD 0.1) in % after 20 min exposure on the differently pre-weathered laminate surfaces with and without deposition of ASW is presented in Figure 8a. Results from parallel control exposures performed on non-pre-weathered sterilized microscopic glass slides are included for comparison to ensure that observed reductions in viability are not predominantly a consequence of dry-out effects of the droplets as this will reduce bacteria viability. A considerable (and statistically significant) reduction (>97%) in viability of *E. coli* was observed for all samples, independent of the extent of pre-weathering or pre-deposition of ASW. A slightly lower antimicrobial effect was observed for surfaces pre-weathered for 1, 2 and 4 weeks with ASW compared to the 1-day pre-weathered surface, though the viability reduction was still high (>96.5%).

Parallel studies conducted on the Gram-positive bacteria *Bacillus* showed similar results with a reduction in viable cells exceeding 99%, even though some of the antimicrobial effects could most probably be attributed to dry-out of the bacteria-containing ASW droplets, Figure 8b, since the bacteria viability also was somewhat reduced on the control sample (glass).

The presence of silver-doped phosphate glass in the resin of the laminate surfaces evidently enabled a considerable reduction in viability of both *E. coli* and *Bacillus* after 20 min of exposure (Figure 8). An additional antimicrobial effect of the TiO<sub>2</sub>-pigment (see experimental) can, though, not be excluded. No viable bacteria were observed on any of the surfaces after 24 h, mainly due to complete droplet dry-out (also on glass control, data not shown). For the powder coated surfaces, no significant reduction in *E. coli* viability could be observed after 20 min of exposure for coating A (the lower concentration of silver-doped phosphate glass), Figure 9a, whereas a reduction was observed for *Bacillus*, Figure 9b, even though some droplet dry-out effects were evident. This shows that the white pigment of the powder coating containing both TiO<sub>2</sub> and BaSO<sub>4</sub> (see experimental) had minor antimicrobial effects.



**Figure 8.** The antimicrobial efficacy (reduction in viability) towards *E. coli* (a) and *Bacillus* (b) after 20 min for the differently pre-weathered (cyclic wet periods at constant temperature, with and without artificial sweat (ASW) deposition) laminate surfaces treated with silver-doped phosphate glass as antimicrobial additive. The results are presented as mean values of duplicate measurements of each condition with a variation between 10–20%.

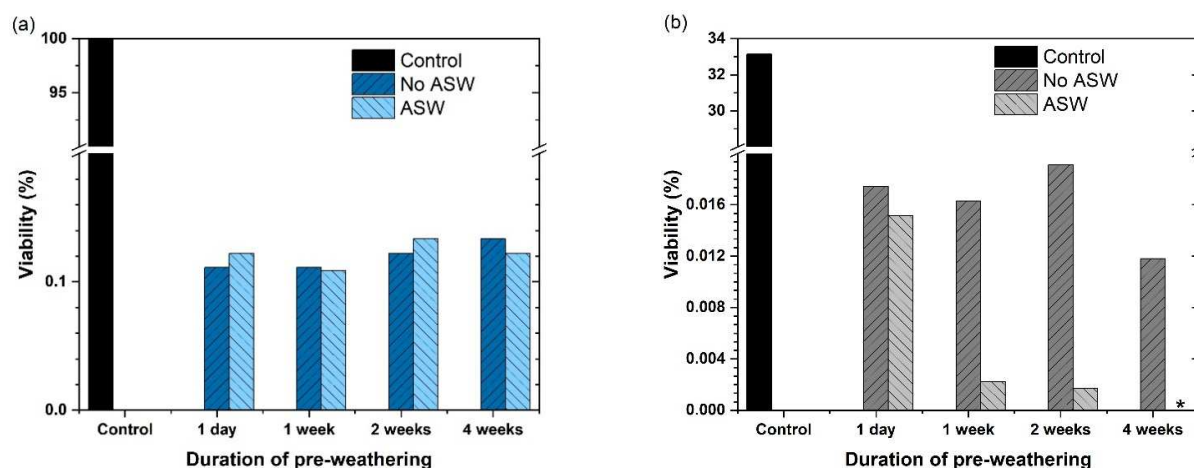


**Figure 9.** The antimicrobial efficacy (reduction in viability) towards *E. coli* (a) and *Bacillus* (b) after 20 min for the differently pre-weathered (cyclic wet periods at constant temperature, with and without artificial sweat (ASW) deposition) powder coated surfaces treated with 0.5 wt.% silver-doped phosphate glass (coating A) as antimicrobial additive. The results are presented as mean values of duplicate measurements of each condition with a variation between 10–20%.

Observed differences in viability between the different bacteria might reflect differences in how released silver ion interacts with the peptidoglycan-rich cell walls of the Gram-negative (*E. coli*) and the Gram-positive (*Bacillus*) bacteria. Gram-negative bacteria have an inner cytoplasmic cell membrane and an outer membrane predominantly composed of lipopolysaccharides, whereas the cytoplasmic membrane of Gram-positive bacteria is surrounded by a thicker layer of peptidoglycan [37]. The considerably thicker (30 nm vs. 3–4 nm) negatively charged peptidoglycan layer of Gram-positive compared to Gram-negative bacteria make them comparably more resistant towards silver ions (which damage the cell walls, and within the cell influence DNA, damage bacterial proteins, and promote the formation of reactive oxygen species), even though the lipopolysaccharide-rich cell membrane of the latter improves its barrier properties [38]. Such differences have been reported in the literature [21,37,38]. Differences in terms of cell membrane thickness, structure, and composition, as well as in the conformation of the peptidoglycan layer, can hence

determine the antimicrobial potency of released silver ions [38,39]. Further investigations, including other bacteria strains, are needed to gain a mechanistic understanding of findings for the commercial laminate and powder coatings investigated in this study.

Powder-coated surfaces with a twice as high concentration of silver-doped phosphate glass in the powder coating (coating B, 1 wt.%) resulted in a considerable reduction in viability of both bacteria, Figure 10. An improved effect was also observed for *Bacillus*, although the effect was predominantly a result of droplet dry-out, as the viability of the bacteria on the control was considerably reduced (>65%).



**Figure 10.** The antimicrobial efficacy (reduction in viability) towards *E. coli* (a) and *Bacillus* (b) after 20 min for the differently pre-weathered (with and without artificial sweat (ASW) deposition) powder coated surfaces treated with 1 wt.% silver-doped phosphate glass antimicrobial additive (coating B, twice as high concentration as for coating A, Figure 9). The results are presented as mean values of duplicate measurements of each condition with a variation between 10–20%.

In all, the antimicrobial efficacy of laminate and powder coatings treated with silver-doped phosphate glass was not hampered by differences in surface weathering at simulated atmospheric indoor conditions and fingerprint contact. The extent to which the silver additive within the powder coatings influenced the antimicrobial efficiency was both material- and bacteria-dependent. Antimicrobial effects attributed to the white pigments consisting of  $\text{TiO}_2$  (in the laminate) and  $\text{TiO}_2/\text{BaSO}_4$  (in the powder coatings) cannot be excluded.

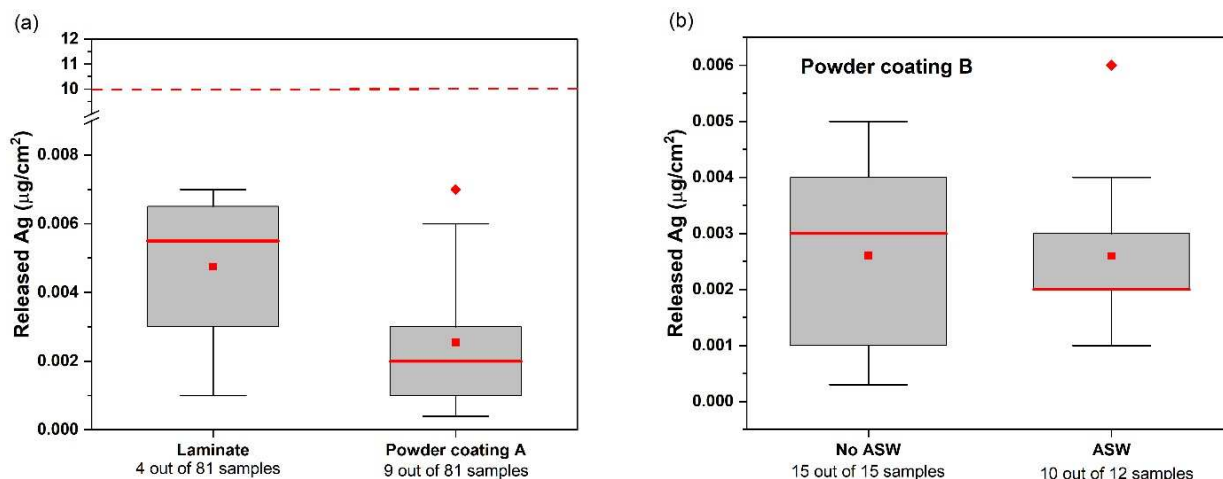
### 3.2. Extent of Release of Ag in Artificial Sweat and Presence of Ag at the Outermost Surface

No silver (less than approx. 1 at.%) was observed in the outermost surface, either by means of XPS (top 5–10 nm) or by means of EDS ( $\mu\text{m}$ -depth information), on any of the laminate surfaces. This is in line with the information provided by the manufacturer (see experimental) and calculated amounts in the materials corresponding to very low amounts of silver from the silver-doped phosphate glass additive in the top laminate layer. No silver was either observed by means of XPS or EDS at the top surface of any of the powder coatings, independent on silver-doped phosphate glass concentration (0.5 or 1.0 wt.%).

The released amounts of silver determined from the laminate and powder coated (coating A) surfaces immersed from 4, 24, and up to 168 h in ASW are presented in Figure 11a. The results show very limited amounts of released silver and observations only from a few replicates (4 out of 81 of the laminate samples and 9 out of 81 of the powder-coated (A) samples). No significant differences in the released amounts of silver between the two materials could be discerned, even though a slightly lower median amount of released silver per surface area was observed for the powder coating compared to the laminate. For the powder coated surfaces with twice the silver phosphate glass concentration (coating B), measurable amounts of released silver were determined from almost all samples (25 out

of 27 samples), Figure 11b. Despite twice as high silver concentration within coating B, the amounts of released silver were very similar as determined for the powder coating A containing less silver. The amount of released silver was further independent of the duration of pre-weathering (data not shown), and no significant effect of deposition of ASW could be observed, Figure 11b.

The observed released amounts of silver were approximately 10 times lower from the laminate and both powder coatings when compared with previously reported release data for bare silver metal particles in artificial sweat ( $0.06\text{--}0.08\ \mu\text{g}/\text{cm}^2$ ) after similar time periods following the same test protocol [40].



**Figure 11.** Extent of released silver (Ag) in artificial sweat (ASW) (LOD  $\approx 0.0003\ \mu\text{g}/\text{cm}^2$  ( $0.2\ \mu\text{g}/\text{L}$ ) and LOQ  $\approx 0.0008\ \mu\text{g}/\text{cm}^2$  ( $0.5\ \mu\text{g}/\text{L}$ )) from differently pre-weathered laminate and powder coated (powder coating A) surfaces (a) and powder coated surfaces with twice the silver phosphate glass content (powder coating B) pre-exposed with and without ASW (b). The grey box represents the interquartile range, IQR (25th to 75th percentile), the solid red line represents the median (50th percentile), the whiskers show the range within 1.5-IQR, the red squares show the mean value, and the red diamonds show outliers. The red dashed line in (a) represents the limit of released silver according to EU regulation 10/2011 for materials and polymers in contact with food [41].

The released amount of silver from the silver-doped phosphate glass powder (not integrated into any resin or matrix) was, after 4 h in ASW, less than 0.2% ( $0.0017\ \mu\text{g}/\mu\text{g}$ ). Considering its measured BET surface area ( $8.225\ \text{m}^2/\text{g}$ ), this amount corresponds to a release of silver of  $0.021\ \mu\text{g}/\text{cm}^2$ . The released amounts of silver per surface area from the laminate and the powder coatings (A, B) were 4–8 times lower.

Assuming a 20 wt.% silver content of the silver phosphate glass present in the laminate (see experimental) in an amount of  $12\text{--}18\ \mu\text{g}/\text{cm}^2$  ( $2.4\text{--}3.6\ \mu\text{g}/\text{cm}^2$ ), the extent of released silver from the laminate and the powder coatings would correspond to 0.08–0.17% of the silver content of the additive. Assuming a 1–5 wt.% silver content, the corresponding levels would be 20 to 4 times lower, i.e.,  $<0.05\%$  of the mass of the additive.

From a health risk perspective, it should be noted that released amounts of silver were furthermore a thousand-fold lower than the limit of released silver stipulated by the EU regulation 10/2011 for materials and polymers in contact with food [41], Figure 11a. No regulatory limit values exist for indoor hygienic surfaces. The small amounts (and concentrations) released from the laminate and powder coating B were evidently sufficient to considerably reduce the viability of the investigated bacteria (see Figures 8–10).

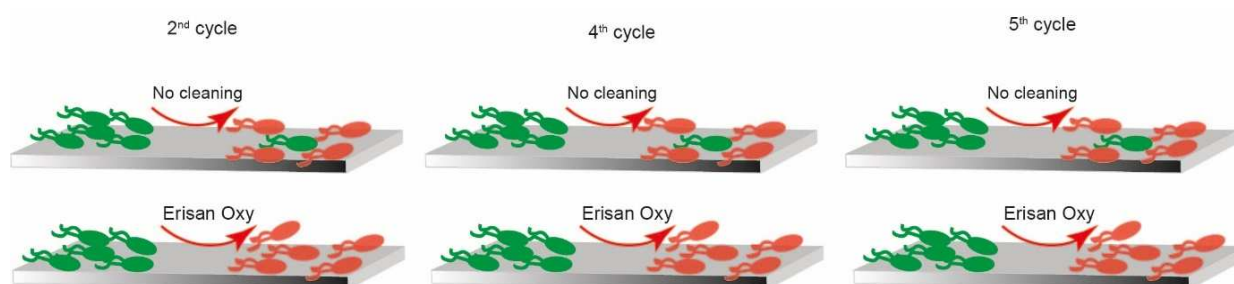
In all, the addition of different concentrations of silver-doped phosphate glass into the matrix of laminate and powder coatings did not result in any detectable amounts of silver at the top surface of any of the materials. However, released amounts of silver in ASW were determined in very low amounts (concentrations), a thousand times lower than

the threshold values stipulated by the regulatory framework for materials and polymers in food contact [41] and approximately in the order of 10 times lower than reported for micron-sized silver metal particles exposed at similar conditions [40].

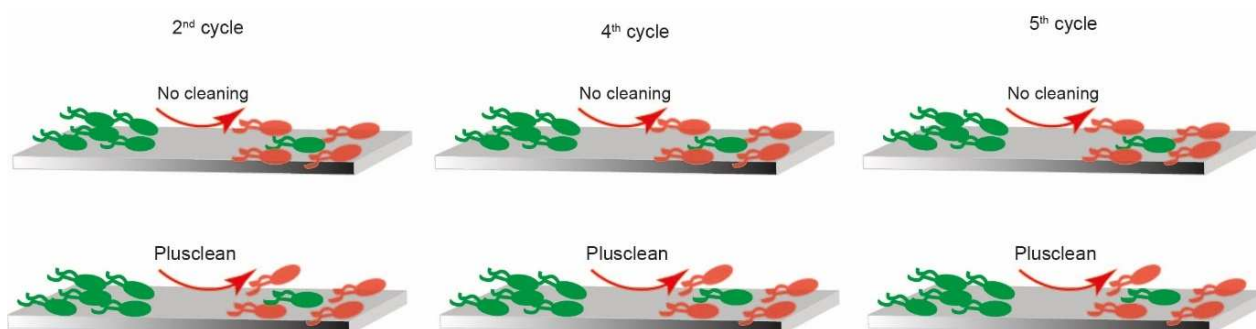
### 3.3. Effect of Repeated Cleaning on Antimicrobial Efficacy

High-touch surfaces in, e.g., health care settings, are daily cleaned with different chemical detergents to hinder the spread of bacteria and viruses. However, the use of such solutions can also influence the material and its surface characteristics, which in turn can influence the antimicrobial efficacy. Results are, in the following, presented to elucidate if manual cleaning using two different commercial detergents (Erisan Oxy+, containing hydrogen peroxide and peracetic acid, and Plusclean, composed of biologically degradable raw materials) is able to hinder bacteria (*E. coli*) growth on silver-doped phosphate glass treated laminate surfaces and whether repeated cleaning influences the antimicrobial efficacy and the surface characteristics.

The cleaning experiments were conducted as described in experimental. Similar to findings after 20 min (see Figure 8), the non-cleaned laminate surfaces were already within 5 min able to reduce the viability of the bacteria with more than 98%, schematically illustrated in Figures 12 and 13. Manual cleaning using both cleaning agents hindered bacteria growth. The effect was even more pronounced (no live bacteria observed) after cleaning with Erisan Oxy+ compared with Plusclean. Some live bacteria were still observed on the surfaces cleaned with Plusclean after the 4th and 5th cycles of bacterial deposition and cleaning, whereas no live bacteria were observed on the laminate surfaces cleaned with Erisan Oxy+ regardless of the cycle of bacterial deposition and/or cleaning.



**Figure 12.** Schematic illustration of the antibacterial efficacy of the laminate surface (pre-weathered for one week) after repeated cleaning with Erisan Oxy+ (5%) and deposition of *E. coli* bacteria (control:  $8.8 \times 10^7$  CFU/mL). Green and red bacteria illustrate live and dead bacteria, respectively.



**Figure 13.** Schematic illustration of the antibacterial efficacy of laminated surfaces (pre-weathered for one week) after repeated cleaning with Plusclean (5%) and deposition of *E. coli* bacteria (control:  $4.8 \times 10^7$  CFU/mL). Green and red bacteria illustrate live and dead bacteria, respectively.

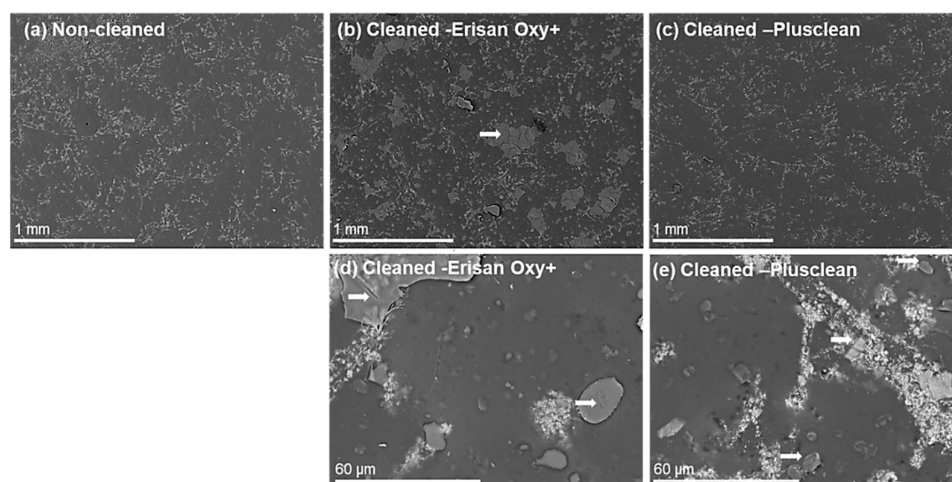
To assess the antimicrobial capacity of the cleaning agents only, droplets of each agent were added onto the deposited bacteria droplets after 5 min, and the presence of live bacteria was determined as described above. Numbers of alive bacteria (in CFU/mL) are

presented in Tables A1 and A2. Surfaces with live bacteria were, in addition, imprinted on NA and the fraction of live bacteria after the cleaning process was calculated, Table 1. The results show that both concentrations of Erisan Oxy+ were sufficient to completely kill the bacteria, whereas some bacteria were still alive on the surfaces when treated with Plusclean.

**Table 1.** Number of live bacteria (in %) after repeated surface cleaning (2, 4 and 5 cycles, see Figure 7) of laminate surfaces using two chemical detergents of different compositions and concentrations (as stipulated by the supplier).

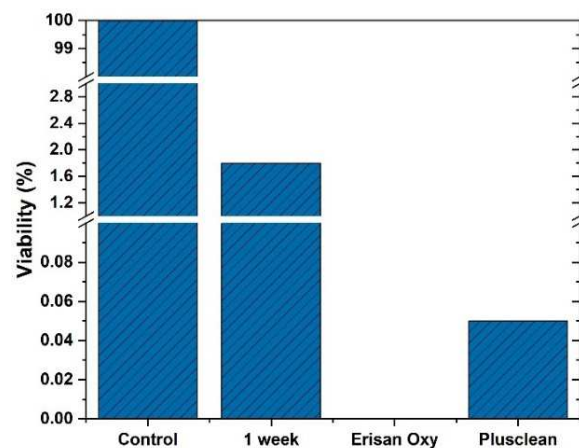
Cleaning Cycle	Plusclean		Erisan Oxy+	
	5 vol.%	10 vol.%	2 vol.%	5 vol.%
2nd	0.003	0.001	0	0
4th	0.008	0.009	0	0
5th	0.019	0.019	0	0

The interactions between the laminate and the cleaning agents did not, according to the SEM investigation, result in any evident differences in the top-surface appearance, neither after cleaning using the biodegradable agent (Plusclean) nor the oxidizing agent (Erisan Oxy+). Evident residues of the cleaning agents, though, remained on the surfaces, see Figure 14, with locally distributed residues of Erisan Oxy+ predominantly composed of oxygen, sodium, and chlorine, and of Plusclean composed of magnesium, silicon, and oxygen. The compositional area measurements with EDS of the laminate matrix (outside the areas with white pigment particles, see Figure 3) revealed no differences in composition with carbon to nitrogen weight ratios of 0.79–0.80 for all surfaces.



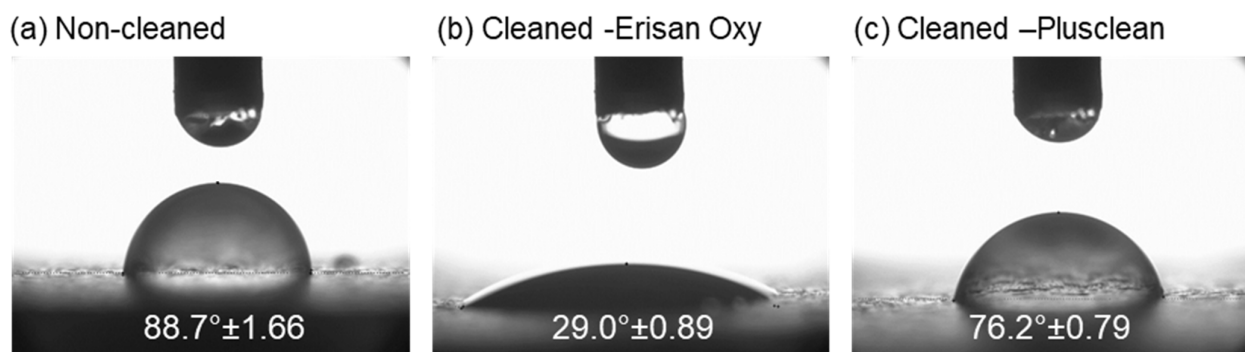
**Figure 14.** Scanning electron microscopy (SEM) images of the top-surface morphology of one-week weathered laminate surfaces before (a) and after 5 cleaning steps using an oxidizing chemical agent (Erisan Oxy+) (b,d) and biodegradable agent (Plusclean) (c,e). The white arrows illustrate some residues from the cleaning agent.

In contrast to complete hindrance and bacteria killing when cleaning the laminate surfaces with Erisan Oxy+, neither repeated cleaning nor interactions with the cleaning agent Plusclean improved the capacity of the laminate surface to hinder bacteria growth compared to non-cleaned surfaces. Similar effects were observed for laminate surfaces repeatedly cleaned 40 times using both cleaning agents before being tested for their antimicrobial efficacy, see Figure 15. The results show that cleaning the laminate surfaces with an oxidizing cleaning agent (Erisan Oxy+) is successful in the complete killing of *E-coli* bacteria, whereas cleaning with the bio-based agent (Plusclean) will not improve the antimicrobial effect.



**Figure 15.** The antimicrobial efficacy after 20 min of laminate surfaces towards *E. coli* including pre-weathered (one week, see Figure 8) and cleaned surfaces (40 times using oxidizing-(Erisan Oxy+) and biobased-(Plusclean) cleaning agents). The results are presented as mean values of duplicate measurements of each condition with variations within 10–20%.

The results are further supported by the presence of residues of the cleaning agents, as this changed the wettability of the surfaces. The effect was more pronounced on the laminate surfaces cleaned with Erisan Oxy+, for which the surface, after repeated cleaning (40 times), became more hydrophilic compared with non-cleaned surfaces (Figure 16). Similar effects, though considerably less pronounced, were observed as a result of cleaning with Plusclean. The results imply an improved antimicrobial effect of cleaned surfaces, both due to the antimicrobial effect of the cleaning agents (in particular, Erisan Oxy+) and due to an increased contact area between the sweat droplets, hence the bacteria with the antimicrobial laminate surface.



**Figure 16.** Static contact angles (wettability) of pre-weathered laminate surfaces prior to (a) and after 40 repeated cleaning cycles using 10 vol.% Erisan Oxy+ (b) and 5% Plusclean (c) cleaning agents.

In all, the results show that the oxidizing chemical agent (Erisan Oxy+) was more efficient in cleaning the surfaces and killing the bacteria, both with and without manual surface cleaning (abrasion), compared to the agent composed of biologically degradable constituents (Plusclean) leaving viable bacteria behind. The non-cleaned laminate surfaces reduced the number of bacteria with >98%. Some bacteria were still alive after 5 min exposure but were completely killed by the oxidizing chemical agent. Surface cleaning resulted in reduced wettability and left residues on the surfaces, effects that are beneficial from an antimicrobial efficacy perspective.

#### 4. Concluding Remarks and Outlook from a Sustainability Perspective

The importance of applying indoor hygiene concepts and using antimicrobial surfaces in public spaces, where many people spend their time or are passing through, to hinder



the spread of bacteria and infections is elucidated in this study. This industry-academic collaborative study assessed the antimicrobial performance of commercial laminate and powder-coated surfaces treated with silver-doped phosphate glass additives in relation to the effects of surface weathering and repeated cleaning at indoor hygiene conditions. The investigated materials are typically used as high-touch surfaces in public areas such as hospitals and schools. Indoor surface weathering and fingerprint contact did not, or only slightly, reduce the antimicrobial properties. The extent of released silver from the surfaces in artificial sweat was orders of magnitude lower than regulated threshold values for silver-containing materials in food contact. An additional antimicrobial effect was observed as a result of repeated surface cleaning using chemical agents of oxidizing properties.

The study is of considerable societal relevance and closely connected to the United Nations Sustainable Development Goals [42], which aims to create comprehensive plans of actions for sustainable development to improve human health and protect the environment. The utilization of antimicrobial materials and coatings for high-touch surfaces is a promising approach in preventing infections from spreading in public spaces. The investigated materials of this study address sustainability aspects in multiple ways and are relevant for both SDG 9 (Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation) and SDG 17 (Revitalize the global partnership for sustainable development). In relation to SDG 3 (Ensure healthy lives and promote well-being for all at all ages), their use is beneficial from a health and well-being perspective by proactively improving the usability and healthiness of shared public spaces and assisting in lowering the costs of health care.

The emerging climate crisis has highlighted the fact that the whole-life impact of buildings is critical in targeting a net-zero carbon-built environment. Sometimes, positive values, such as energy savings and hygiene, can become adversarial with one another; for instance, if the water temperature in the plumbing network is lowered too much, it creates a favorable environment for microbial growth. In this study, the comprehensive indoor hygiene concept, elucidated by hygienic surfaces with antimicrobial properties, highlights the importance of considering the whole life cycle of a building (SDG 12 Ensure sustainable consumption and production patterns). Hence, a holistic approach and communication between different actors are needed when bringing innovative solutions to the construction industry since it involves many actors and interactions at multiple levels. An important aspect that needs more attention in the future is related to recycling and waste management of antimicrobial materials containing, e.g., silver, at the end of the life cycle. More multidisciplinary research and industry–academia collaboration are needed to further improve sustainability. Users and producers of the materials should discuss and implement the results. Users of antimicrobial materials also need to be involved in discussions of environmental aspects in order to implement the results of this study in the most efficient way.

**Author Contributions:** Conceptualization, I.O., E.B. and M.U.; Methodology, I.O., E.B., G.H., G.K.R.; Validation, I.O., E.B. and G.H.; Formal Analysis, I.O., G.H. and G.K.R.; Investigation, I.O., G.H., G.K.R. and E.B.; Resources, I.O. and E.B.; Data Curation, I.O. and E.B.; Writing—Original Draft Preparation, I.O., E.B., M.A., R.M. and G.H.; Writing—I.O. and E.B., Review & Editing, I.O., M.A., R.M., G.H., M.U., T.M., T.M. (Tiina Mäkitalo) and E.B.; Visualization, I.O., G.H., T.M. (Tuomas Mehtiö) and E.B.; Supervision, I.O. and E.B.; Project Administration, I.O., T.M. (Tiina Mäkitalo) and E.B.; Funding Acquisition, I.O., M.A., R.M., T.M. (Tiina Mäkitalo) and E.B. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Number of live bacteria (CFU/mL) after cleaning the laminate surface using Erisan Oxy+. The deposited number of bacteria was  $8.8 \times 10^7$  CFU/mL.

Number of Cleaning Cycle	Live Bacteria (CFU/mL)	
	No Cleaning	Cleaning
2nd	$3.5 \times 10^4$	0
4th	$5.6 \times 10^4$	0
5th	$4.4 \times 10^4$	0

**Table A2.** Number of live bacteria (CFU/mL) after cleaning the laminate surface using Plusclean. The deposited number of bacteria was  $4.8 \times 10^7$  CFU/mL.

Number of Cleaning Cycle	Live Bacteria (CFU/mL)	
	No Cleaning	Cleaning
2nd	$1.9 \times 10^4$	0
4th	$1.5 \times 10^4$	12
5th	$1.4 \times 10^4$	57

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