

ORIGINAL ARTICLE

Investigation of the effectiveness of antimicrobial photocatalyst-coated hand-contact surfaces in passenger transport vehicles under everyday conditions

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Abstract

The coronavirus disease-2019 pandemic affects all aspects of public life. Measures for infection prevention are implemented in various sectors, in businesses, as well as in private life. Public transport is important and indispensable in daily life for both children and adults.

Public transport companies have to take necessary actions to protect passengers and drivers from infections. Skin contact is one of the ways of transmitting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

This research study was designed to evaluate the effectiveness of a photocatalytic, antimicrobial active surface coating under everyday – not hospital – conditions. To date, such coatings have been used in hospitals as an additional measure to regular cleaning and disinfection in order to reduce the risk of infection.

We collected samples for bacterial cultures in three classes of public transport vehicles: bus, underground, and tram. Seven different hand-contact surfaces in one vehicle of each class were coated, while the other vehicles remained uncoated. All vehicles were in regular use. The number of colony-forming bacterial units per cm² (CFUs/cm²) was measured. A representative number of isolates were differentiated at the pathogen level. Data collected were entered into GraphPad Prism (GraphPad Software, San Diego, USA) and analyzed.

Overall, no statistically significant reduction in the number of colony-forming units (CFUs) was observed for coated versus uncoated surfaces. Samples with a very high colony count (>250 CFU/25 cm²) were equally distributed in both groups, coated and uncoated vehicles. Within one vehicle type, there was no significant difference between the coated and the uncoated vehicle. No relevant infection-preventive effect could be proven.

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Local public transport is indispensable in many aspects of public life. Users have contact with various surfaces in these vehicles while traveling. Skin contact is one of several ways of transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We investigated the impact of a photodynamic active antimicrobial surface coating in public transport vehicles.

Background

According to current research, about 90% of SARS-CoV-2 is transmitted via droplets and aerosols, while only about 10% transmitted by physical contact (1, 2). In vehicles of public transportation (bus, underground, and tram), passengers are often not able to maintain social

distancing due to the type of construction. Furthermore, possibilities of ventilation are limited. Limiting the number of passengers is possible, but difficult to control and enforce. Data on infections acquired by means of public transport are non-existent. International governmental organizations have published recommendations for the prevention of infections in public transport (3–6).

On the one hand, economic damage can be caused to the individual (e.g. caused by illness and quarantine). On the other hand, economic damage can be caused to the companies of public transportation (e.g. shortfall caused by decreasing numbers of passengers), as well as to the society (caused by increasing numbers of COVID-19-infections) (7). Moreover, macroeconomic losses are caused by the absence of staff members due to sickness

(8, 9). The implementation of hygiene measures also results in additional financial expenses that have not been necessary before. Several international studies and articles have described the aforementioned effects. The first study concerning these effects was published in China in line with the pandemic. These effects have also been reported in other areas of international transport, including container and cargo transport (10).

Ruhrbahn, the public transport company established in Essen, Germany, has, therefore, decided to test the coating of hand-contact surfaces for its vehicles as one of several ways for the protection of passengers and drivers. The coating tested was Dyphox® Universal (TriOptoTec GmbH, Regensburg), a product developed under the leadership of the University of Regensburg. This coating can be applied to components using various techniques (e.g. spraying and lacquering). Under the visible light conditions (wavelength 400–700 nm), by a catalytic process it produces so-called singlet oxygen from ambient oxygen (11, 12). In type II reactions, the photodynamic active substance that is included in the applied coating transfers energy to molecular oxygen and generates highly reactive singlet oxygen (1O_2). The singlet oxygen photo-oxidizes biomolecules such as lipids and proteins, leading to lysis of cell membranes (13), thereby killing bacteria and viruses. The sphere of action of this singlet oxygen is about 1 mm.

The producer recommends the use of Dyphox® Universal coating as an additional hygiene measure to maintain a low microbial load between two cycles of disinfection. The intention is to complement, not replace, manual surface disinfection and hand disinfection (14). Dyphox® Universal specifications state an effect at a temperature range from below 0°C to over 45°C, with natural visible light or artificial light (wavelength 400–700 nm) (14). The efficacy of the product was proven in a study under controlled laboratory conditions in two hospitals (15). This study showed an advantage of coating. It reduced samples with a high-pathogen load (>2.5 or >5 CFU/cm²). The values reported above this limit is assessed as an aspect relevant to infection (16).

The spectrum of activity of Dyphox® Universal includes different types of bacteria (e.g. *Staphylococcus aureus* or *Escherichia coli*), enveloped and non-enveloped viruses, or fungi and multi-resistant germs (14). The new beta-coronavirus, SARS-CoV-2, is an enveloped RNA virus from the same family as severe acute respiratory syndrome (SARS) and MERS. Its properties allow a long survival on surfaces (16, 17). The duration of this survival depends, among other factors, not only on the material but also on the humidity of these surfaces (18, 19).

The approach to prevent the transmission of pathogenic microorganisms via the coating (20) or treatment of surfaces (21) or the use of different materials with

corresponding properties is not a novelty. In the hospital sector, there has been no gold standard available, so far (22, 23, 24). Here, the focus is on certain human pathogens, which are particularly responsible for nosocomial infections (11, 12, 15).

We investigated the impact of a photodynamic active antimicrobial surface coating in public transport vehicles. The aim of this study was to find out whether the coating with Dyphox® Universal would have a bacteria-reducing effect under everyday conditions, especially considering non-optimal lighting conditions, rapidly successive and very frequent contacts, and the absence of intermediate disinfection during the operating time. Furthermore, it was used to find out whether a significant infection-preventive effect could be expected.

Methods

Two vehicles in each of three vehicle classes (bus, underground, and tram) were tested. Prior to the time of the study, no regular disinfection in the passenger area was practiced. After ending operations in the evening, the vehicles were cleaned by cleaning teams without subsequent disinfection.

As the incidence of COVID-19 was low at the time of the study in Essen, Germany, detection of SARS-CoV-2 was not the objective of this study. Therefore, the decision was, as in the study on the efficacy of Dyphox® Universal in hospital, to choose a surrogate criterion. For this purpose we chose the change in bacterial growth, respectively, the killing of bacteria expressed by the number of colony-forming bacterial units per cm².

Influencing factors

The intention was to exclude or minimize factors that might influence the effectiveness of coating. These factors include the influence of light (hours of sunshine, artificial light, light incidence, and intensity of sunlight), light intensity, temperature, humidity and number of passengers.

Light: To optimize the photocatalytic effect under daily routine, all artificial lights in the vehicles remained switched on permanently until the samples were taken. The light intensity was not measured because of the permanently changing conditions during the journey. A continual measurement of light intensity on each sampling site in all the vehicles did not seem to be practical. Instead, all vehicles were tested under identical light and weather conditions on the same days and operating time periods.

Temperature: The temperature may also be of variable influence. The daytime temperature was obviously not influenceable; however, the vehicles were all tested on the same day, so that the results under the same conditions should be comparable. The exact influence of temperature on the sequence of this special catalytic process is unknown.

Humidity: The ambient humidity and the hand-contact humidity could influence the photocatalytic process, as well as the survival of microorganisms. All vehicles were tested on the same days, so the results could be compared.

Number of passengers: Varying passenger numbers, as well as the differential frequency of skin contacts, result in different loads of contamination. Therefore, the same route was always taken and the drivers logged the number of passengers.

One of the special features was the passenger usage in the underground: two wagons were coupled together, one of which had been treated and the other left untreated. At the final destination, the travelling direction was changed to ensure all parts of the vehicles had been equally used.

Owing to the shape of the sampled areas, standardized tools (pressure strength and contact duration) could not be used. In order not to keep people from not touching the uneven surfaces, we decided to leave these unmarked. We decided not to use swabs because the measurement of the total amount of germs was the primary aim. Consequently, the samples on the handrails were taken in a rolling motion. Samples were taken on the same day by the same person in each of the treated and untreated vehicles to guarantee a constant sequence of events.

The samplers were trained in this technique, as well as in the preparation of samples, including storage and transportation. The samples were labeled before being taken for analysis to prevent confusion. Because the risk of infection in public transport does not just apply to the passengers but also apply to the drivers, and hence, one sample was also taken from the driver's area.

The numbers of drivers, which varied between three and four per day (average 3.68 drivers/day), could also have an influence on the impact factor of microbial colonization. No statistically significant difference in drivers' numbers could be observed between treated and untreated vehicles.

The hand-contact surfaces in one of each of the vehicles under study were coated with photodynamic effective Dyphox® Universal. The hand-contact surfaces in the other vehicle remained uncoated to function as a control group. The amount of bacterial growth was measured. Dyphox® Universal, a photocatalyst coating, was newly applied to all regularly used hand-contact surfaces in three vehicles according to manufacturer's specifications (laquered as a paint finish) at the start of this study.

Surfaces in the vehicles with frequent contact were selected. In the passenger area, these were the vertical bars at the entrance (entrance is upward; therefore, this help is often used). The same is applied to the grab handles between the seating groups and the horizontal grab bar in the area of the severely handicapped or disabled seating areas, which are frequently touched when getting up and sitting down. In the exit area, this included

the stop request button, and the vertical bar on which the switch is mounted (Figure 1). The exterior contact surfaces of vehicles were deliberately excluded, as currently all doors at each stop open automatically without request. In the driver's area, the equipotential bonding grip was selected for tram and underground vehicles and a fixed area of the operation monitor in buses; the criterion was the aspect of frequent use.

Documentation of the sampling points was prepared with numbering, photograph, and description. Also, each component was marked with a sticker with the corresponding number on the side to avoid confusion. Per vehicle, six samples were taken from the passenger area and one sample taken from the hand-contact area of the driver.



Figure 1. Example of sampling item – stop call button.

In all vehicles, the entire components were wipe-disinfected after sampling (mikrozid® universal wipes premium, Schülke & Mayr GmbH, Norderstedt, Germany). The reaction time was waited for and, at the end of the entire sampling, a negative control in both trams (coated and uncoated) was carried out on the same component to control disinfection. The date and time of sampling were documented, as well as the proper condition of the sampling plates (Rodac plates), also shift start, shift end, number of drivers, weather conditions, and passenger revenue.

The samplers were trained, and the samples were stored in temperature-controlled refrigerators, collected, and prepared the next morning. An insulated Dyphox® Universal-coated vehicle restraint bar was also experimented on. This pole was first disinfected, and then uniformly contaminated with common skin germs and sampled at zero time and two sites after 2, 5, 10, 20, 30, 60, 120, and 4,320 min. The pole was continuously exposed to natural ambient light without artificial illumination.

Microbiologic methods

The measurement of bacterial colony-forming units (CFUs) was based on sampling performed with RODAC plates with Columbia sheep blood agar (Oxoid Deutschland GmbH, Wesel, Germany). Incubation and evaluation were performed by the Eurofins laboratory (Eurofins Laborbetriebsgesellschaft Gelsenkirchen, Department of Hospital Hygiene). All measured values were converted to CFU/cm². A representative number of samples (the same number in coated and uncoated trams) were differentiated at the pathogen level. The determination was performed by laser spectrometry (MALDI-TOF Biotyper MBT smart, Bruker Daltonik GmbH, Bremen, Germany; database version BDAL-IVD rev. 9 2019-11-04 + IVD Extension rev. 1 2019-04-01). The laboratory did not know whether the samples came from a coated or an uncoated vehicle.

Statistical analysis

The results of CFU counts were tested for normal distribution using the Shapiro–Wilk test ($p < 0.0001$, $\alpha = 0.05$) and the Kolmogorov–Smirnov test ($p < 0.0001$, $\alpha = 0.05$). As there was no normal distribution, the results were evaluated using the Mann–Whitney test, two-sided, for unrelated samples. In addition, mean, median, and standard deviation values were determined. The absolute and relative risk for high bacterial counts (> 2.5 and > 5 CFU/cm²) and the odds ratio (95% confidence interval) were also determined. A value of $p < 0.05$ was considered to be statistically significant. Analysis was conducted using the software PRISM (GraphPad Software LLC, San Diego, CA, USA; Version 8.4.3 for Mac).

Results

All documents were available and could be evaluated. All vehicles could be sampled as planned; two vehicles were out of operation for 1 day each. The passenger revenue corresponded to the regular number; there were no differences in passenger revenue between coated and uncoated vehicles. The weather conditions were identical for all vehicles. The average daily temperature was estimated to be 22°C. The lowest daily temperature was 11°C; the maximum temperature was 33°C. Regarding light, all vehicles drove under identical conditions; the average number of sunshine hours per day was 10.4 h, with a minimum value of 4.6 h and a maximum value of 14.4 h. The average driving time was 794 min.

The sample results seem plausible; there are only a few samples observed with very high values. However, this result declares itself from the whole-surface contact (palm). The samples with a very high colony count (> 250 CFU/25 cm²) were equally distributed in both groups. The pathogens found were largely normal skin germs and germs of the surrounding flora (e.g. coagulase negative *staphylococci*, *Micrococcus*, and aerobic spore-formers). However, few others are relevant as hospital germs. In contrast, the disinfection-control samples showed no

Table 1. Colony-forming units (CFUs)/cm² per vehicle

Vehicle	Bus coated	Bus uncoated	Tram coated	Tram uncoated	Underground coated	Underground uncoated
Median	1.360 <i>n</i> = 70	1.620 <i>n</i> = 56	1.339 <i>n</i> = 70	1.160 <i>n</i> = 70	1.360 <i>n</i> = 77	1.480 <i>n</i> = 77
Mean	2.138	2.409	1.982	1.588	2.208	2.529
Standard deviation	2.153	2.409	2.129	1.737	2.309	2.642
Maximum CFU/cm ²	10	10	10	10	10	10
Minimum CFU/cm ²	0	0	0	0	0	0
<i>P</i> -value	0.6748		0.2361		0.4484	
Significant difference ($p < 0.05$)	No		No		No	

Test procedure: Mann Whitney U, two-sided

CFU per vehicle (coated/uncoated) Mean and Standard deviation

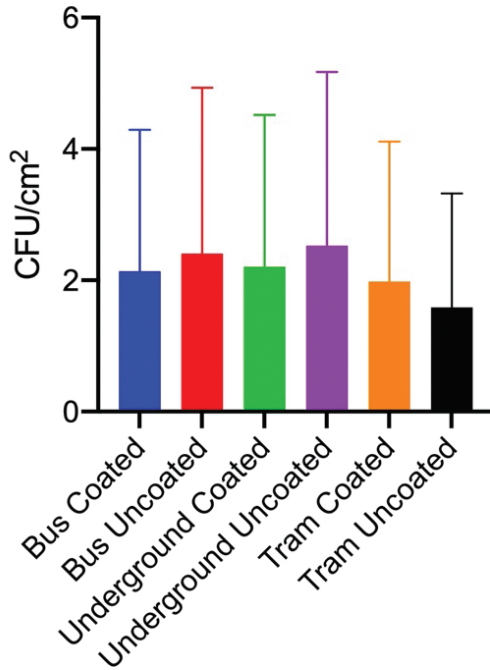


Figure 2. Colony forming units (CFUs)/cm²: mean and standard deviation per vehicle class.

bacterial growth after disinfection, with two exceptions (0.16 and 0.04 CFU/cm²).

The mean value of CFUs, in general, was 2.113 CFU/cm² on coated and was 2.171 CFU/cm² on uncoated surfaces; however, the values did not differ significantly (Mann-Whitney test $p = 0.8790$), $p < 0.05$). The detection of high bacterial counts (> 2.5 or > 5 CFU/cm²) also showed no significant difference. The number of CFUs looking at single-vehicle classes on coated or uncoated vehicles did not differ significantly (all: $p > 0.05$), ranging from 1.588 to 2.642 CFU/cm² (Table 1 and Figure 2).

At test locations 1 (handrail, vertical, at exit), 3 (stop call button at exit), 4 (holding loop), 5 (handrail, vertical, middle of vehicle), and 6 (grab handle between seats), no significantly higher number of CFUs on one vehicle type was observed. Test location 2 (handrail, vertical, under stop call button) presented a significantly higher number of CFUs on coated vehicles. Test location 7 (contact plate in driver’s cabin) showed a significantly higher number of CTUs on uncoated vehicles (Table 2 and Figure 3). At location 3 (stop call button at exit), the highest bacterial counts were observed (Figure 4).

Under everyday conditions, such as the number of passengers, the average use of hand-contact surfaces (handrails, grab handles, and control buttons), and

Table 2. Colony-forming units (CFUs)/cm² per sampling point

Test location	1 coated	1 uncoated	2 coated	2 uncoated	3 coated	3 uncoated	4 coated	4 uncoated	5 coated	5 uncoated	6 coated	6 uncoated	7 coated	7 uncoated
Median	1.24 n = 31	1.24 n = 29	2.24 n = 31	0.96 n = 29	4.54 n = 31	3.32 n = 29	2.00 n = 31	1.56 n = 29	0.68 n = 31	0.88 n = 29	1.32 n = 31	1.16 n = 29	0.44 n = 31	1.00 n = 29
Mean	1.88	1.98	2.04	1.55	4.66	4.42	2.21	2.51	1.01	1.17	2.06	1.64	0.93	1.94
Standard deviation	2.39	2.21	1.18	1.61	1.18	1.60	1.77	2.25	1.02	1.05	1.99	1.32	1.74	2.61
Maximum CFU/cm ²	10	10	4.72	7.2	10	10	10	10	4.04	3.6	10	4.56	10	10
Minimum CFU/cm ²	0	0.04	0.2	0.16	0.19	0.36	0.16	0	0	0.04	0.12	0	0	0
P-value	0.6835		0.0275		0.4964		0.8978		0.5153		0.6461		0.0488	
Significant difference ($p < 0.05$)	No		Yes		No		No		No		No		Yes	

Test procedure: Mann-Whitney U, two-sided

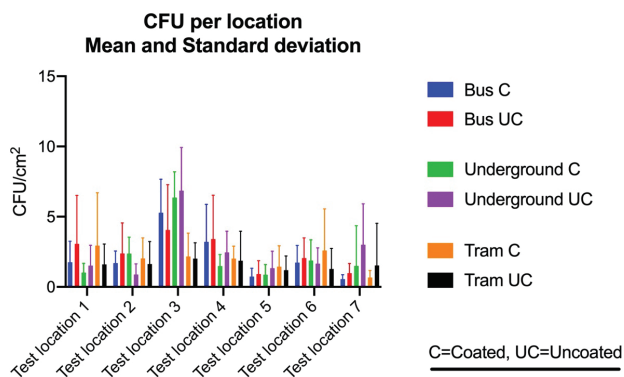


Figure 3. Colony-forming units (CFUs)/cm² by sampling location and vehicle.

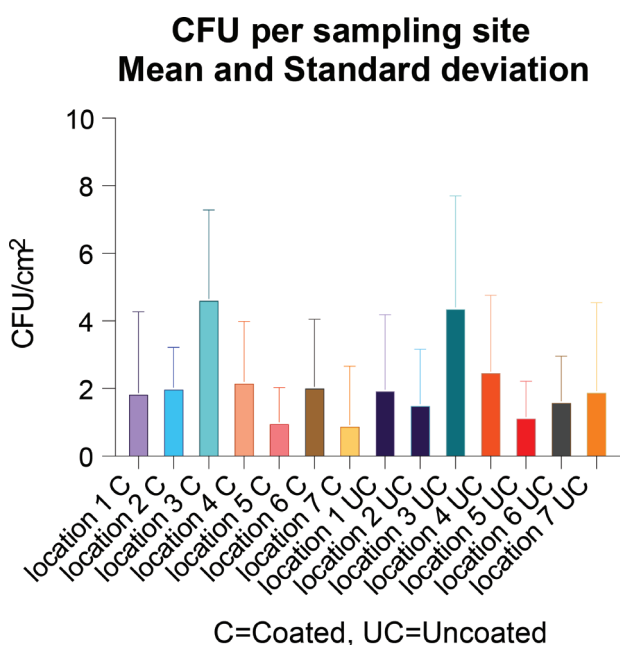


Figure 4. Colony-forming units (CFUs)/cm² by sampling site.

environmental conditions (e.g. light incidence, temperature, etc.), no significant difference in the amount of bacterial growth on hand-contact surfaces could be observed when comparing coated with uncoated surfaces in the vehicles and types of public transport.

In the experiment for estimating the minimum light irradiation time required for a relevant effect, a reduction in the number of CFUs showed up after 60–120 min at the earliest.

Discussion

This study was explicitly designed to test real contamination caused by physical contact in vehicles used daily, generated under representative conditions of regular operation, including, for example, existing light sources and daylight illumination.

The total number of CFUs detected was very low, which is lower than expected in non-pandemic situations. This makes it more difficult to identify relevant differences and certainly does not represent the situation in pre-pandemic times.

Overall, no statistically significant reduction in the number of CFUs was observed for coated compared with uncoated surfaces. Within one vehicle type, there was also no significant difference between coated and uncoated vehicles.

At a single sampling point, the difference was significantly reduced in favor of the coated surface and at another single sampling point in favor of the uncoated surface. Thus, no relevant infection-preventive effect was observed across all surfaces.

It should be noted that only limited time was available for this experiment, as the results were needed on time to decide on the further procedure of establishing safety precautions. Accordingly, the power of the study is sufficient to prove medium effects (Cohen *d* = 0.5).

Under these conditions, no relevant differences between coated and uncoated surfaces could be observed in the test setup. In a pandemic, low contamination may also be a consequence of passengers’ caution, who hold on as little as possible or operate control buttons even with aids or protected by clothing, or even use hand disinfectants they carry with them.

We believe, however, that one of the main reasons for this result could be the exposure to light, which is not optimal under everyday conditions and necessary for the process, due to insufficient light intensity, and the fact that the light is blocked by hand contact. We also consider the short time between contacts to be a relevant factor. The photodynamic process only takes place in the time between two covers or contacts; in the time of light closure it stops.

To summarize, no relevant infection-preventive effect could be proven. Overall, this explicitly does not speak against the general effectiveness of coating surfaces but only against the advantage of coating with Dyphox® Universal over uncoated contact surfaces in this special setting with passenger use under given environmental conditions. According to current findings, as the proportion of contact transmission of COVID-19 is also significantly lower than that of aerosol or droplet transmission, other measures to prevent infection in public transport should also be considered (25).

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Declaration of conflicting interests

The authors have declared that they have no conflict of interest regarding this manuscript.

Ethics approval

Ethics approval is not applicable.

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