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Micro-textured films for reducing microbial colonization in a clinical setting

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SUMMARY

Background: Transmission of microbes in the hospital environment occurs frequently through human interactions with high-touch surfaces such as patient beds and over-bed tables. Although stringent cleaning routines are implemented as a preventive measure to minimize transmission of microbes, it is desirable to have high-touch surfaces made of antimicrobial materials. Physical texturing of solid surfaces offers a non-bactericidal approach to control the colonization of such surfaces by microbes.

Aim: To investigate the efficacy of micro-textured polycarbonate films in reducing bacterial load on over-bed tables in a hospital ward.

Methods: Two different micro-patterns were fabricated on polycarbonate film via a thermal imprinting method. Micro-textured films were then mounted on patient over-bed tables in a general hospital ward and the bacterial load monitored over 24 h. Total colony counts, which represented on-specific bacterial loading, and meticillin-resistant *Staphylococcus aureus* counts were monitored at each time-point.

Findings: Over a period of 24 h, both micro-textured surfaces showed consistently lower bacterial load as compared to the unpatterned polycarbonate and the bare over-bed table laminate. This study supports the findings of earlier laboratory-scale studies that micro-scale physical texturing can reduce bacterial colonization on a solid surface.

Conclusion: Results of the current study suggest that micro-textured surfaces could provide a viable method for reducing microbial contamination of high-touch surfaces in hospitals.

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Introduction

Hospital-acquired infection (HAI) remains a challenge worldwide, with an estimated global prevalence rate of 3–21% [1]. Hospital environments act as reservoirs for healthcare-associated pathogens, notably, meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci,

Clostridium difficile, and *Acinetobacter* spp. Such pathogens transmit between patients and their environment through healthcare workers or directly through 'high-touch' surfaces such as bed rails, bed surfaces, supply carts, drip poles, and over-bed tables [2].

Good hand hygiene remains the most efficacious method of HAI control [3]. However, compliance is generally low, with approximately only 30% in general wards [4,5]. Hand hygiene is practised mostly before and after patient interaction but less so after contact with patient surroundings [4]. High contact frequency with such surfaces imposes a challenge in

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implementing stringent hand hygiene; therefore, environmental cleaning and disinfection remain important [6].

One emerging solution is the use of antimicrobial materials. For example, furniture could be manufactured or coated with silver nanomaterials and copper-based alloys; however, these materials are costly and have potential side-effects [7,8]. For instance, silver nanomaterials raise toxicity concerns due to leakage, whereas copper-based alloys suffer from corrosion issues.

Bacterial colonization depends on both chemical and physical surface characteristics. Whereas chemical-based antimicrobial solutions are commercially available, there is continued effort to develop new approaches to address the evolving resistance of microbes to bactericides [9]. One potential non-chemical approach is micrometre- and nanometre-scale surface texturing. Surface topographies influence cellular activities such as adhesion, proliferation, and phenotype modulation, and may undermine biofilm formation by disrupting bacterial attachment, growth, and colonization [9–11].

Lithography is used to fabricate well-defined surface topography which enables the systematic investigation of the effect of surface topography on bacterial behaviour. For example, high aspect ratio nanostructured silicon showed a six-fold decrease in viability of *Escherichia coli* and *S. aureus*, and inhibition of *E. coli* attachment on micro-patterned polyethylene terephthalate in early stages [12,13]. Previous studies also observed reduction in bacterial attachment on microscale silicon honeycomb structures [14] and polydimethylsiloxane with microgrooves [15]. However, other studies have reported that surface patterning showed an enhanced bacterial colonization [16,17]. Although the mechanistic aspects behind the bacterial responses on patterned surfaces remains elusive, there is encouraging evidence demonstrating the potential of surface texturing in microbe control.

However, most reported studies have been performed under well-controlled conditions using fixed parameters such as inoculation concentration, culture medium, and temperature, which do not represent dynamic and uncontrolled real-world conditions. For example, it is known that hydrodynamic conditions in an aqueous medium influence bacterial attachment [13]. Bacteria on dry surfaces show different viabilities to planktonic bacteria, which are more characteristic of studies performed under wet laboratory conditions. In a hospital ward setting, however, surfaces change between dry and wet conditions unpredictably. High-touch surfaces also have a dynamic bacterial load; they are exposed to microbes from human touch, foreign objects, and ambient air, while also subject to disinfection routines, resulting in variable exposure conditions and bacterial load. Therefore, the conventional laboratory approach is not representative of hospital wards [18].

A recent study simulated the touch surface in hospitals by inoculating a bacterial culture on to the test sample through a soaked cloth [19]. However, this exposure method still fails to account for the uncontrolled exposure conditions from patients, visitors, hospital personnel, and the varying environmental conditions that may affect bacterial colonization.

This study investigates the effects of well-defined micro-textured film surfaces on bacterial contamination, when such surfaces are placed on high-touch surfaces in a hospital ward.

Methods

Fabrication and characterization of micro-textured polycarbonate films

Nickel moulds were silanized using vacuum-assisted vaporization of 0.1 mL 1H-1H-2H-2H-perfluorododecyltrichlorosilane (Alfa Aesar, Ward Hill, MA, USA) for 4 h in a desiccator: (i) 10 µm cylindrical pillar with 1:5 duty cycle and 1:2 aspect ratio (Eulitha AG, Kirschdorf, Switzerland); and (ii) 1.5 µm (peak-to-peak) V-groove grating with groove height of 2 µm (NIL Technology ApS, Kongens Lyngby, Denmark).

Moulds were then imprinted on 5 × 5 m² polycarbonate films (PC2151; Innox HIGA Singapore Pte Ltd, Singapore) at 40 bar and 190°C for 10 min (SOLVES TI60.250.4) and cooled to room temperature before demoulding.

Samples were gold-coated (JEOL JFC-1600 Auto Fine Coater) and pattern fidelity was confirmed via JEOL JSM-7600F scanning electron microscopy (SEM).

Static water contact angles (WCAs) of 3 µL H₂O droplets on the surfaces were measured using a goniometer (Kino, Norcross, GA, USA) and the Young–Laplace fitting method in CAST3.

Hospital field testing

Field testing was conducted in a geriatric ward in Changi General Hospital, Singapore. The two test patterns and an unpatterned polycarbonate were assembled on a polycarbonate template, which was then mounted on an over-bed table (Supplementary Figure 1). All test surfaces were cleaned with isopropanol wipes before mounting and then again with isopropanol and Mikrozid[®] AF disposable wipes (Schülke & Mayr Asia, Singapore) after mounting.

Bacteriological sampling

Fifty templates were mounted on 50 over-bed tables, 25 of which were being used for MRSA-positive patients. Sampling was performed over a 24 h period. Each test surface was swabbed with flocked swabs at predetermined time-points: after mounting ($t = 0$ h), before lunch ($t = 4$ h), before tea ($t = 8$ h), and before breakfast ($t = 24$ h). Normal ward activities remained unaffected; for instance, tables were wiped down with a wet rag after meals and then disinfected with Mikrozid spray (23% ethanol, 35% 1-propanol) and disposable wipes.

Sampling was carried out by rolling the swab across the test surface ten times line-by-line. Swabs were eluted separately in 0.5 mL D/E Neutralizing Broth (BD Difco, Franklin Lakes, NJ, USA) and vortexed for 20 s; 0.1 mL of the eluate was inoculated in 5% Typticase-Soy Sheep Blood agar (BD BBL[™] TSA II; BD Difco) in duplicates. From a 1:10 dilution, 0.1 mL was also inoculated into a separate plate, also in duplicates. These steps were repeated with chromogenic MRSA-selective agar (MRSA-Select, BD BBL[™] CHROMagar[®] MRSA II; BD Difco) for samples from MRSA-positive patients. Plates were incubated overnight at 37°C under ambient air conditions. Total colony counts (TCC) and MRSA colony counts (MRSA-TCC) were assessed by visual inspection.

Statistical testing

TCC and TCC-MRSA data were assessed for normality by Anderson–Darling test, then analysed by Wilcoxon test or *t*-test (Igor Pro). For each test sample, a pairwise comparison was performed against the bare control surface.

Results

Film fabrication and characterization

Micro-textured polycarbonate films were fabricated through thermal nanoimprinting: 10 μm cylindrical micropillars and 2 μm V-grooves. Structure fidelity and uniformity were examined under SEM. Samples achieved nearly 100% imprinting yield. Micropillar polycarbonate structures showed an appreciable degree of tapering that is uniform across the sample. V-groove polycarbonate structures were well-replicated uniformly across the entire sample (Figure 1).

The micropillar polycarbonate film surface was super-hydrophobic with a static WCA of 162° , although water droplets wet down occasionally over time (Table I). On the other hand, the V-groove polycarbonate surface showed anisotropic wetting properties with WCAs of 92.8° along the grating axis and 134° perpendicular to it (Supplementary Figure 2).

Bacteriological sampling

Total colony counts and MRSA colony counts as a function of film exposure time are shown in Figures 2 and 3.

Table I

Static water contact angle on micro-textured polycarbonate films

Test surface	Water contact angle ($^\circ$)
Pristine (unpatterned) polycarbonate	98.3 ± 4.6
Cylindrical micropillar polycarbonate	162.1 ± 3.3
V-groove polycarbonate (perpendicular to grooves)	134.0 ± 1.7
V-groove polycarbonate (parallel to grooves)	92.8 ± 3.4

Surfaces with water contact angle $>120^\circ$ are strongly hydrophobic, whereas $>150^\circ$ is considered super-hydrophobic.

Statistical analysis

Graphs of TCC data sets at $t = 24$ h (Supplementary Figure 3) suggest that these data sets were not normally distributed, which is confirmed by the Anderson–Darling test (Table II).

TCC data sets at $t = 24$ h were analysed non-parametrically by Wilcoxon test. A pairwise comparison was performed against the original table (control) surface. The mean TCC and standard deviation results are reported in Table II, and comparative statistical results are reported in Table III. The flat surfaces (pristine polycarbonate and control) exhibited the highest mean colony counts. There was no significant difference between the two surfaces ($P = 0.9$).

The textured surfaces both exhibited a lower mean bacterial count (Table II). Pairwise comparisons against the control table surface revealed that this difference in bacterial levels was statistically significant ($P < 0.05$) (Table III).

Figure 1. Scanning electron micrographs of imprinted polycarbonate films: cylindrical micropillar polycarbonate (MP-PC) cross-section (A); V-groove polycarbonate (VG-PC) cross-section (B); overhead MP-PC (C); and overhead VG-PC (D). Scale bars = 10 μm .

Figure 2. Total colony counts as a function of film exposure time, with one standard deviation. MP-PC, cylindrical micropillar polycarbonate; VG-PC, V-groove polycarbonate.

Figure 3. Meticillin-resistant *Staphylococcus aureus* (MRSA) colony counts as a function of exposure time, with one standard deviation. MP-PC, cylindrical micropillar polycarbonate; VG-PC, V-groove polycarbonate.

Anderson–Darling tests performed on MRSA-TCC data sets at $t = 24$ h showed that control, micropillar polycarbonate, and V-groove polycarbonate surfaces were normally distributed, whereas the pristine polycarbonate surface was non-normally distributed.

Dependent t -tests were performed for pairwise comparisons of the test surfaces (Table III), and a significant difference ($P = 0.05$) was found between the micro-textured surfaces and the control surface at a critical threshold. The observed significance between the control surface and the micropillar polycarbonate film endures at the more stringent $P = 0.01$.

Differences in the performance of micropillar and V-groove polycarbonate films, if any, are unclear, as comparative testing revealed no statistically significant difference between the two data sets.

Discussion

Both surface textures were fabricated on free-standing polycarbonate films, a polymer with relatively high modulus and glass transition temperature; it is used in a wide range of

Table II

Anderson–Darling tests of test surfaces for total colony counts (TCC) and meticillin-resistant *Staphylococcus aureus* colony counts (TCC-MRSA) at $t = 24$ h

Surface	TCC				TCC-MRSA			
	Mean	SD	Test statistic	P-value	Mean	SD	Test statistic	P-value
Control	151.70	138.13	3.14	<0.0050	56.235	24.139	0.41	0.3082
Pristine	153.50	138.39	3.12	<0.0050	59.471	24.288	0.91	0.0156
MP-PC	109.04	100.55	3.25	<0.0050	36.471	14.257	0.32	0.4960
VG-PC	110.98	116.17	3.15	<0.0050	34.647	22.773	0.25	0.7184

MP-PC, cylindrical micropillar polycarbonate; VG-PC, V-groove polycarbonate.

Table III

Comparative statistical tests of test surfaces for total colony counts (TCC) and meticillin-resistant *Staphylococcus aureus* colony counts (TCC-MRSA) at $t = 24$ h

Compared surfaces		TCC		TCC-MRSA	
Surface 1	Surface 2	Test statistic	P-value	Test statistic	P-value
Control	Pristine	1231.5	0.8990	0.3896	0.699
Control	MP-PC	1580	0.0224	2.9068	0.007
Control	VG-PC	1552.5	0.0366	2.6822	0.010
MP-PC	VG-PC	—	—	0.3982	0.348

MP-PC, cylindrical micropillar polycarbonate; VG-PC, V-groove polycarbonate.

consumer products and is easily handled through known industrial processes. This scalability makes it an ideal test material for study as it could easily be adapted for commercial use. Whereas the selection of these textures was guided by prior laboratory-scale studies, the current work represents a performance study. Although some ward activities are controlled and routine (such as meal times and cleaning schedules), there is a range of highly unregulated events, such as patient interaction with hospital personnel and visitors, patient movement within or outside the ward, and environmental factors such as local humidity and temperature. Such uncontrolled phenomena introduce variations in bacterial loading between patients and therefore account for the high variation within the data sets.

Micropillars are a common texture in several antimicrobial studies; such arrays have been reported to exhibit higher WCAs compared with corresponding pristine surfaces. In cases where WCAs $>150^\circ$ were measured, such surfaces were considered super-hydrophobic. One study reported that when the pillar size was $<1\ \mu\text{m}$, a higher degree of bacterial reduction was observed; another study showed reduction on pillars $>10\ \mu\text{m}$, though to a smaller extent [20]. Although the design principle behind the pillar structure has not been established, from the fabrication perspective, lithography costs for resolutions $<1\ \mu\text{m}$ are substantially higher than for resolutions $>1\ \mu\text{m}$. Hence, the $10\ \mu\text{m}$ round pillar and $2\ \mu\text{m}$ V-groove geometries were chosen for this study.

Scanning electron micrographs show that the $10\ \mu\text{m}$ round pillars have a re-entrant profile even though the mould has cylindrical wells. This effect could be attributed to post-imprinting demoulding; as the pillars are subjected to tensile force, deformation could result in a thinner pillar 'neck'. The re-entrant profile could also account for the high WCA values.

Differences in surface wetting could explain the effect of topography on bacterial colonization. Although there are yet no conclusive findings on the antimicrobial effect of surface hydrophobicity, a number of studies have reported that super-hydrophobic surfaces mitigate bacterial attachment [12]. In this study, test samples are dry hydrophobic films with high WCAs. Compared to a flat surface, textured surfaces generally trap a thin air layer resulting in a composite surface consisting of solid and air interface; such a composite surface might be an obstacle to bacterial adhesion and colonization.

The differences between the mean TCC of textured surfaces and non-textured surfaces were most pronounced at $t = 24$ h, whereas they were much more similar at earlier time-points. Since tables are cleaned after every meal, there is typically only 4 h of incubation between $t = 0$ h, $t = 4$ h, and $t = 8$ h. At $t = 24$ h (breakfast), however, 12 h have passed after the previous table cleaning (dinner), thereby leading to higher TCCs and amplifying the effects of textured surfaces at $t = 24$ h.

Further statistical analyses of TCC and TCC-MRSA at $t = 24$ h show that the differences between textured surfaces and bare table laminate are significant at $P = 0.05$. Hence, we conclude that both textures are effective in reducing the colonization of microbes compared to the bare over-bed table surface. However, whereas both textures exhibit lower TCC than the table laminate, V-groove polycarbonate has a lower WCA than micropillar polycarbonate and exhibits anisotropic wetting behaviour. This would suggest that surface hydrophobicity is not a key reason for the observed reduction in TCC on V-groove polycarbonate. The V-groove structure, however, presents a constricted topography that may restrict bacterial colonization. Textures restricting the spreading of attached bacteria through physical constraint have been reported previously [21].

Further studies should be conducted to investigate the underlying mechanisms governing the interaction between bacteria and these textures. Understanding how these geometries restricted bacterial contamination—whether through suppressing adhesion, proliferation, or even detachment during sampling—might inform future novel designs to achieve greater effects.

Despite the large standard deviation, trend lines and comparative statistical analyses demonstrate the efficacy of these micro-textured surfaces in reducing bacterial colonization on meal tables at $t = 24$ h. Furthermore, despite obvious structural and wetting differences between micropillars and V-groove structures, both textures show similar performance

in minimizing bacterial colonization on meal tables. As aforementioned, the mechanism behind TCC reduction is non-chemical; instead it is speculated that this is accomplished primarily through physical constraint, which restricts fouling.

One strength of our methodology is that it studies the performance of these textures on the actual bacterial flora and environmental conditions in a hospital ward. As mentioned in the Introduction, previous controlled studies have shown a correlation between surface textures and bacterial morphology in determining bacterial colonization, and the proposed mechanisms are varied. More importantly, it is unclear how a mixed load of bacterial species with various morphologies and surface affinities would interact with topography and with each other and whether such interactions might affect colonization. Designing laboratory-scale studies to simulate real-world conditions is a challenging task and is likely to be limited by the variation and unpredictability that the real world provides, such as inoculation of a multi-species bacterial load with species concentrations varying with time.

Physical texturing is a non-chemical approach to minimizing bacterial colonization on a solid surface, free from environmental and health concerns associated with chemical-based antimicrobial solutions. In this study, textured films were fabricated through nanoimprinting, a scalable nanofabrication technique for film-based products. Besides nanoimprinting, surface texture can also be fabricated by the injection moulding process that is used in the manufacture of many products, including hospital furniture. For this technology to be viable, it is important to ensure mechanical durability against scratch and abrasion. Generally, micrometre and nanometre surface textures retain the intrinsic mechanical properties of the bulk materials. In other words, scratch- and abrasion-resistant textures may be achieved by selecting scratch- and abrasion-resistant materials.

In conclusion, there is growing interest in hospital furniture, appliances, and accessories made from antimicrobial or microbe-resistant materials. Our report offers a pragmatic approach to studying the efficacy of novel solutions to combating HAI by integrating inevitable real-world uncertainties such as environmental variation and interpersonal interaction into the experimental design. Instead of relying on biocidal chemical coatings, the two micro-textures that we investigated present a physical constraint to microbial colonization. After 24 h, both micro-textures had consistently lower bacterial (TCC and TCC-MRSA) loads on over-bed tables in Changi General Hospital. Polycarbonate can be easily and economically adapted to include such surface microstructures. This scalability allows for the feasible use of micro-textured polycarbonate films in reducing microbe colonization in hospitals to combat the enduring problem of HAI.

This study presents a real-life performance test, the results of which support the findings of earlier laboratory-scale studies that physical texturing in the micro- and even nano-scale dimensions may reduce bacterial colonization on a solid surface.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jhin.2017.08.001>.

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