Polymeric paint coated common-touch surfaces that can kill bacteria, fungi and influenza virus

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Abstract

In the current situation of COVID-19 pandemic, the role of surfaces in transmitting pathogens is clearer than ever. Herein, we report an organo-soluble, quaternary antimicrobial paint (QAP) based on polyethyleneimine (PEI) which was coated on a wide range of surfaces such as polyvinylchloride (PVC), nylon, rubber, aluminum. The coating completely killed drug-resistant bacteria. It showed rapid bactericidal properties with complete killing in 45 min of exposure and lowered bacterial adherence, asserting self-sterilizing nature. The coating exhibited complete killing of stationary phase cells of bacteria. The coating killed drug-resistant *C. albicans* strains. Importantly, QAP coating showed complete killing of influenza virus (H1N1).

Introduction

Community or hospital acquired infections (HAIs) have become a major threat leading to a significant number of deaths worldwide.^[1–3] In spite of the quality of the medical infrastructure, onset of healthcare associated infections has posed a tremendous burden on patients increasing the medical expenses as well as the risk of morbidity.^[4] Health care-associated pathogens are prevalent in the patient environment and are known to transmit by deposition on wide range of inanimate surfaces such as bed rails, door knobs, medical equipment, tables, etc.^[5,6] Such common-touch surfaces, upon touch by a healthy individual, may spread the pathogens thereby triggering notorious infections.^[7–9] As practiced currently, regular cleaning/disinfection of patient environment are expected to lower the risk of HAIs. However, such activities require the application of toxic chemicals, manpower and training of personnel.^[10,11]

In the recent past, scientific community has progressed significantly in developing antimicrobial coatings for surfaces to tackle fomite-associated infections.^[12–15] Cationic antimicrobial polymers have emerged as a desired candidate for the development of self-sterilizing surfaces.^[16] Several groups have developed covalent grafting techniques to attach antimicrobial polymers comprising quaternary ammonium moieties to different surfaces.^[17–19] In another approach, researchers have developed an alternative method to coat surfaces through physical deposition or non-covalent attachment of cationic polymers.^[20–25] Our group has reported the development of an organo-soluble, hydrophobic, quaternary polyethyleneimine (PEI)-based antimicrobial paint. In a prototype study, these polymers were coated on glass slides and their antibacterial and antifungal activities were evaluated.^[20]

In this study, we have synthesized an organo-soluble, quaternary antimicrobial paint (QAP) based on linear polyethyleneimine (PEI) backbone with octadecyl hydrophobicity and fabricated the coating on a wide range of surfaces, such as nylon, poly(vinylchloride), rubber and aluminium. The coated surfaces were characterized through scanning electron microscopy. The antibacterial activity of the coated surfaces was checked against drug-resistant Gram-positive and Gram-negative pathogens. The surfaces were also investigated for their potency against bacteria-laden aerosol through spraying technique. QAP coated surfaces were checked for their bactericidal kinetics against superbug MRSA. The ability of the surfaces to reduce the adherence of bacterial cells was also examined. To assess the ability of the QAP coating to kill human pathogenic fungi, their antifungal activity was checked against *Candida* spp. Importantly, QAP coated surfaces were also checked for their efficacy to kill human influenza virus (H1N1).

Materials and methods Chemicals and instrumentation

Reagent grade dichloromethane (DCM), chloroform (CHCl₃), tert-butanol (tBuOH), ethanol (EtOH) and methanol (MeOH) were obtained from Spectrochem (India). HPLC grade isopropanol (IPA) was procured from Spectrochem. Dimethyl sulfoxide (DMSO) and poly(2-ethyl-2-oxazoline) were purchased from Sigma Aldrich. Drying of solvents was done wherever necessary. 1-bromooctadecane was purchased from Sigma Aldrich. The chemicals were directly used for reaction. Nuclear magnetic resonance (NMR) spectra of the compounds were recorded in deuterated solvents in a Bruker AMX-400 spectrometer. PVC, nylon and rubber sheets of thickness 0.3 mm

and aluminium sheet (0.4 mm thickness) were bought from local hardware vendor. Methicillin-resistant *Staphylococcus aureus* (MRSA R3545), *E. coli* R3336 and *P. aeruginosa* R590 were obtained from the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. Fungal strains (*C. albicans* AB226 and *C. albicans* AB399) were obtained from Anthem Biosciences, Bangalore, India. As a solid growth medium, nutrient agar was used for both Gram-negative and Gram-positive bacteria. YPD agar was used for fungi-related experiments. Influenza virus A/NWS/33 (H1N1) was procured from ATCC (Rockville, MD, USA). The 96-well plates and 6-well plates were obtained from Tarsons (India). Plasma was obtained by centrifugation and discarding the red blood cells donated by a healthy donor at Jawaharlal Nehru Centre for Advanced Scientific Research. SEM and EDAX were performed in Zeiss Gemini 500 FESEM comprising an EDX unit.

Synthesis of hydrophobic cationic polymer

Synthesis of deacylated linear PEI^[20]

Fully deacylated linear PEI was prepared by the hydrolysis of poly(2-ethyl-2-oxazoline) (PEOZ) (50 kDa) in presence of hydrochloric acid as acid catalyst. Briefly, 10.0 g of PEOZ was added with 400 mL of 24% (wt/vol) HCl and the mixture was refluxed for 96 h (Fig. 1). After initial dissolution of the PEOZ crystals, a white precipitate appeared. After 96 h of stirring, the white precipitate was filtered using G3 sintered glass funnel followed by air-drying. The linear PEI precipitate was subjected to deprotonation by dissolution in 50 mL water. Consequently, aqueous 6 M KOH solution was added until the pH of the solution was ~11. The curd white precipitate of the deprotonated PEI was filtered using a G1 sintered glass

Figure 1. Quaternary antimicrobial paint (QAP). (i) Synthetic scheme of QAP, (ii) Characterization through (a) IR spectroscopy; (b) and (c) ^1H -NMR spectroscopy of N-methyl PEI and QAP respectively, (iii) Scanning electron microscopy images of QAP coated surfaces (A: polyvinylchloride, B: rubber, C: nylon and D: aluminium) and elemental mapping for bromide of QAP coated surfaces (E: polyvinylchloride, F: rubber, G: nylon and H: aluminium).

funnel. This precipitate was then repeatedly washed with distilled water until the pH was ~ 7 . After complete deprotection, 4.4 g of linear PEI (22 kDa) was yielded.

Synthesis of linear *N*-methyl PEI

Deprotonated PEI (4.4 g) was transferred to a round-bottom flask. To this flask, 90% formic acid (24.5 mL) was added followed by 37% formaldehyde (29.3 mL) and 20 mL of water (Fig. 1). The reaction mixture was allowed to stir at 90°C for 60 h. The mixture was then cooled to room temperature. KOH solution (8 M) was added to the mixture until the pH of the solution was ~ 11 . The deprotonated *N*-methylated PEI was extracted several times by chloroform. The entire organic solution was then subjected to repeated water wash. Chloroform was then removed to yield a yellow viscous product with 100% degree of methylation. $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ): 2.242 (s, 3H, $-\text{N}(\text{CH}_3)-$), 2.491 (s, 4H, $-\text{N}(\text{CH}_2\text{CH}_2)-$).

Synthesis of *N*-Octadecyl, *N*-Methyl PEI

N-methyl PEI (2.4 g, 42 mmol of repeating unit) was dissolved in 30 mL of tert-butanol in a screw-top pressure tube and 1-bromooctadecane (168 mmol) was added to the solution (Fig. 1). The reaction mixture was heated at 120°C for 96 h in an oil bath. After completion of the reaction, the solvent was reduced using a rotary evaporator under reduced pressure. An excess of diethyl ether (~ 150 mL) was added to the reaction mixture and precipitation of pale brownish product was observed. The precipitate was filtered off and washed with acetone. Re-dissolution in CHCl_3 was performed and diethyl ether was added to reprecipitate the product for further purification. The excess ether was then decanted and the precipitate was dried using a high vacuum pump to yield brownish yellow *N*-octadecyl *N*-methyl PEI polymer (QAP) with 95% isolated yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ): 0.871 (t, 3H, terminal $-\text{CH}_3$), 1.249–1.539 (br m, 30H, $-(\text{CH}_2)_{15}$), 1.796–1.863 (m, 2H, $-\text{CH}_2-\text{CH}_2\text{N}^+(\text{CH}_2)_2(\text{CH}_3)$), 3.005–4.236 (m, 9H, $-(\text{CH}_2)_2\text{N}^+(\text{CH}_2)(\text{CH}_3)$).

Solubility of *N*-octadecyl, *N*-methyl PEI

Solubility of the polymer was checked by dissolving 10 mg of the compound in 1 mL of wide range of solvents: chloroform, dichloromethane, methanol:DMSO (1:1), chloroform:DMSO (1:1). The solubility was determined visually.

Coating of surfaces and characterization

QAP was coated on a wide range of surfaces from its organo-solution. 10 mg/mL solution of QAP was prepared in CHCl_3 :DMSO (1:1). PVC, rubber, nylon and aluminium surfaces were cut into $2 \times 2 \text{ cm}^2$ pieces. The pieces were then washed thoroughly in isopropanol to remove any impurity. Further washing was performed in water and acetone. After drying

the surfaces, 200 μL of the organo-solution was dropped casted on them. The surfaces were then dried to form a uniform film.

Field emission scanning electron microscopy

QAP coated surfaces (PVC, rubber, nylon, aluminium) were characterized through field emission scanning electron microscopy (FESEM) to evaluate the surface morphology. The samples were sputtered with gold prior to imaging.

Energy dispersion X-ray

Energy dispersive X-ray (EDX) analysis was performed for the QAP coated surfaces in Zeiss Gemini 500 FESEM comprising an EDX unit to analyze the presence of elements on the surfaces. All the samples were sputtered with gold before experimentation.

Antibacterial activity of coated surfaces through dragging on agar plates Against planktonic cells

The activity of the surfaces was tested following our previously published protocol.^[19] Clinically isolated drug-resistant strains of MRSA and *E. coli*, namely MRSA R3545 and *E. coli* R3336 were grown in nutrient broth for 6 h at 37°C with constant shaking to obtain mid-log phase bacterial cells. The bacterial solutions were then diluted in saline to attain a cell concentration of $\sim 10^6$ CFU/mL. 10 μL aliquot of this suspension was dropped on a $2 \times 2 \text{ cm}^2$ surfaces (PVC, nylon, rubber and aluminium) coated with QAP. Uncoated surfaces were used as negative controls. The surfaces were incubated for 40 min after dropping of bacterial suspension, which was followed by dragging them on nutrient agar plates. The nutrient plates were then incubated for 18 h at 37°C followed by imaging of the plates. Every sample was investigated in triplicate.

Against stationary cells

A mid log phase MRSA R3545 and *E. coli* R3336 cultures were diluted ~ 1000 times in nutrient growth media incubating it at 37°C for 16 h under constant shaking condition. Post-incubation, the bacterial cells were centrifuged down at 9000 rpm for 2 min and resuspended in saline to achieve a concentration of $\sim 10^6$ CFU/mL. This suspension was used for further experiment following similar protocol as that of investigation of antibacterial activity against planktonic cells. Every sample was investigated in triplicate.

Antibacterial activity of coated surfaces through visual turbidity

MRSA R3545 and *E. coli* R3336 were harvested in mid-log phases by growing for 6 h in suitable nutrient media at 37°C

with continuous shaking. The bacteria were then diluted in saline in order to prepare a suspension of $\sim 10^6$ CFU/mL. 10 μ L of this suspension was dropped on 2×2 cm² surfaces (PVC and nylon) coated with QAP. Uncoated surfaces were used as negative controls in the experiment. After 40 min of incubation at 37°C, the surfaces were dropped into freshly prepared nutrient broth (10 mL) individually followed by incubation for 18 h. The tubes were then checked visually for any turbidity and photographed.

Antibacterial activity against droplet-borne bacteria

The experiment was performed following a previously published protocol.^[20] 2×2 cm² nylon and PVC surfaces were used for investigating the efficacy of QAP coating to kill bacteria when exposed to pathogen-laden aerosols. Uncoated surfaces were used as negative controls in the experiment. MRSA R3545 and *P. aeruginosa* R590 were harvested from a 6 h culture in the mid-log phase. The bacteria were diluted in 10% plasma (in saline) to obtain a concentration of $\sim 10^6$ CFU/mL. The bacterial suspensions were sprayed on the surfaces using a TLC sprayer at a spray rate of 10 mL per minute. After spraying, the surfaces were left for drying for 30 min followed by rubbing on nutrient agar plates. The plates were then incubated at 37°C for 18 h and images were captured.

Bactericidal kinetics of the coated surfaces

Nylon surfaces coated with QAP were used for examining the bactericidal kinetics of the coating. Pristine nylon surfaces were used as negative controls. Briefly, 10 μ L of $\sim 10^6$ CFU/mL suspension of MRSA R3545 was dropped on 2×2 cm² nylon surfaces (coated and uncoated) and incubated at 37°C. At time points 0 min, 20 min and 45 min, the surfaces were washed with 990 μ L of saline. The wash solutions were then serially diluted and 20 μ L of the solutions were dropped on nutrient agar plates. The plates were incubated for 18 h at 37°C for 18 h followed by counting the bacterial colonies.

Bacterial adherence on the coated surfaces

A mid log phase (6 h grown) culture of MRSA was diluted to attain a concentration of $\sim 10^5$ CFU/mL in nutrient broth which was supplemented with 1% glucose and 1% NaCl. Coated and uncoated aluminium and PVC surfaces were used for this experiment. The surfaces were kept immersed in 3 mL of this bacterial suspension for 4 days at 37°C to allow formation of biofilm on the surfaces. Then the surfaces were gently dipped in saline and the adhered bacteria were harvested using 2 mL trypsin–EDTA for every sample. The trypsin solutions were then serially diluted and plated on nutrient agar plates.

The plates were incubated at 37°C for 18 h and colonies were counted.

Antifungal activity of the coated surfaces

Fungal strains (*C. albicans* AB226 and *C. albicans* AB399) were streaked on YPD agar plates followed by incubation at 28°C for 24 h. A single fungal colony was inoculated in 3 mL of YPD media and kept under shaking condition for 10 h at 37°C to attain mid-log phase cells with concentration of $\sim 10^8$ CFU/mL. Coated and uncoated PVC, rubber, nylon and aluminium surfaces were employed in this experiment. These cultures were then diluted in saline to obtain a suspension of $\sim 10^6$ CFU/mL. The procedure followed thereafter was similar to that mentioned in antibacterial assay through dragging method.

Antiviral activity of the coated surfaces

The antiviral activity of QAP coating was investigated against human influenza virus A/NWS/33 (H1N1) through performing plaque assay following a previous report.^[19] 2×2 cm² PVC surfaces were used in this experiment, either uncoated or coated with QAP. In brief, both coated and uncoated PVC sheets were placed in a polystyrene petri dish. A 10 μ L droplet of virus ($\sim 2.4 \times 10^6$ PFU/mL) suspended in PBS was dropped on the surfaces. The surfaces were then covered with an uncoated substrate of similar dimension to effect the spreading of droplet. The surfaces were then incubated at room temperature for 30 min and then washed with 990 μ L PBS thoroughly to withdraw all the viral cells. In case of the uncoated surfaces, this wash solution was 100 fold diluted which was directly used for plaque assay (Well 1) followed by two fold serial dilution for five times (well 2–well 6). These dilutions were then used for infection of a monolayer of Madin–Darby canine kidney (MDCK) cells. However, for coated surfaces, the wash solution was directly used for plaque assay (well 1) along with serially diluted by two folds for five times (well 2–well 6).

Plaque assay

MDCK cells (2 mL, 5×10^6 cells per well) were seeded in 6-well plates at 37°C in a humidified-air atmosphere (5% CO₂/95% air) for 24 h till the cells attained $\sim 95\%$ confluency. The medium was removed and the cells were washed with PBS for two times. After washing the cells, they were infected using 200 μ L of the virus solutions. The plates were then incubated at room temperature for 1 h with occasional rocking of the plates to avoid their drying. The solutions were then removed and the cells were overlaid with 2 mL of plaque media with oxoid agar followed by incubation at 37°C for 72 h. After 72 h, 1 mL of 37% formalin solution was added to every well for fixation. The agars were discarded and crystal violet solutions were added followed by washing with water. The plates were then photographed.

Results and discussion

Synthesis of the quaternary antimicrobial polymer (QAP)

To develop an organo-soluble polymer to be used as an antimicrobial paint, linear polyethyleneimine (PEI, 22 kDa) was chosen as the polymeric backbone. Protected acylated polyethyleneimine (50 kDa) was hydrolyzed under acidic condition to obtain a deprotected amine moiety (Fig. 1). Obtained as a protonated white precipitate, it was deprotonated by treatment with KOH solution to yield linear PEI (22 kDa). Then PEI was subjected to E. Clarke methylation to attain tertiary amine-containing *N*-methylated polyethyleneimine. The complete methylation was confirmed through ^1H -NMR spectroscopy and IR spectroscopy. IR spectrum showed absence of peaks at 1600 cm^{-1} corresponding to the N–H bond bending (Fig. 1). For the final quaternization step, *N*-methylated PEI and an excess amount of 1-bromooctadecane was dissolved in $^t\text{BuOH}$ in a sealed tube, and reacted at an elevated temperature of 120°C for 96 h (Fig. 1). The hydrophobic quaternary polymer (QAP) was precipitated using anhydrous diethyl ether. The product was a brownish precipitate. No unquaternized moiety could be observed in the ^1H -NMR spectrum with the disappearance of peaks between 2.0 and 2.5 ppm (Fig. 1).

Coating of surfaces and characterization

QAP exhibited high solubility (up to the tested concentration of 25 mg/mL) in a wide range of organic solvents including dichloromethane, chloroform, and chloroform:dimethyl sulfoxide (1:1). In an attempt to mimic painting of surfaces, organo-solution of QAP was prepared in CHCl_3 :DMSO (1:1) with a concentration of 10 mg/mL , which was then drop-casted on different surfaces. To include surfaces which occur regularly in our community and healthcare settings, $2 \times 2\text{ cm}^2$ of various surfaces of such as PVC, rubber, nylon, aluminium were coated by drop-casting $200\text{ }\mu\text{L}$ of QAP solution to obtain a surface coverage of $500\text{ }\mu\text{g/cm}^2$. Nylon and aluminium surfaces showed a change in appearance with a pale brownish colour. However, no change in appearance could be observed for coated PVC and rubber surfaces. Coated surfaces were visualized through scanning electron microscopy. A rough film formation was observed on all the surfaces, with a smoother film observed in case of rubber and aluminium substrates. EDX analysis followed by color mapping showed uniform distribution of bromine on the surface, which is present as a counter ion of quaternary moiety of QAP, implying homogeneous coating formation (Fig. 1(iii)).

Antibacterial activity of coated surfaces

Against planktonic bacteria

Community-acquired or nosocomial infections result from the circulation of drug-resistant pathogens in the community or healthcare environment. These microbes adhere to different

inanimate surfaces which upon touch by a healthy individual may lead to the transmission followed by consequent infections. To gauge the efficacy of the QAP coated surfaces to kill notorious bacterial cells, MRSA R3545 and *E. coli* R3336 were chosen as representative Gram-positive and Gram-negative pathogens respectively. Both were clinically isolated from patients and resistant to different conventional antibiotic treatment. Suspensions of these pathogens were dropped on the surfaces coated with QAP [Fig. 2(a)].

After an incubation period of 40 min, the surfaces were dragged on nutrient agar plates to examine the presence of any live bacteria. In case of uncoated surfaces, a thick lawn of bacteria was seen, for all the surfaces tested. However, no bacterial growth was observed on the nutrient agar plates. To further substantiate the claim, the nylon and PVC surfaces, both coated and uncoated, were incubated with freshly prepared bacterial growth medium, post-incubation with bacteria [Fig. 2(b)]. It was observed that in case of uncoated surfaces, the nutrient media turned turbid thereby affirming the presence of live bacterial cells on the surfaces. On the other hand, the media remained clear for coated surfaces, which confirmed the bactericidal efficacy of the coated surfaces with an ability to display 100% killing [Fig. 2(b)].

Against stationary bacterial cells

One of the alarming problems in the healthcare settings is the prevalence of the stationary cells of bacteria, which remain metabolically inactive, thereby surpassing the barrier offered by traditional antibiotic therapeutics. Therefore, upon withdrawal of antibiotic treatment, these stationary cells may lead to recurrence of infections. The surfaces coated with QAP were therefore investigated for their efficacy against stationary cells of MRSA and *E. coli*. Uncoated surfaces showed the presence of a sizeable amount of live bacterial cells, in case of both rubber and aluminium. On the contrary, when the aluminium or rubber pieces were coated with QAP, no bacterial growth could be observed on the nutrient agar plates thereby asserting the efficiency of the coating to completely kill dormant bacterial cells, which are known to evade the action of market-available antibiotics [Fig. 2(c)].

Antibacterial activity against droplet-borne bacteria

One of the major transmitters of pathogens in the hospital settings is the bioaerosol, consisting of particles which remain prevalent in the environment for a long period of time. Such particles have an extended time of flight, and potency to travel substantial distances prior to gravitational settling/deposition on any surface. Such particles are generated through numerous human activities, such as breathing, talking, coughing, sneezing, etc. Even surgical procedures generate a significant proportion of aerosol/droplet particles which may act as a source of pathogens. Different infections can result from the

Figure 2. (a) Antibacterial activity of QAP coated surfaces through dragging of surfaces on nutrient agar plates. (b) Antibacterial activity of QAP coated surfaces through dipping in nutrient media. (c) Antibacterial activity of QAP coated surfaces against stationary phase bacterial cells. Arrow shows the direction dragging.

deposition of such particles on the inanimate common-touch surfaces. With an aim to prevent the transmission of droplet-borne infections, QAP coated surfaces were investigated for their efficacy to kill pathogens when exposed to bacteria-laden fluid particles. Suspensions of MRSA or *P. aeruginosa* in human plasma were sprayed on coated and uncoated surfaces followed by dragging on nutrient agar plates with the help of a tweezer. Thick bacterial lawns were observed for both the bacteria, when their plasma-suspensions were sprayed on uncoated surfaces [Fig. 3(a)]. On the other hand, the QAP coated surfaces showed no growth of bacteria. This observation suggested the efficiency of QAP coating to kill bacteria, upon exposure to a pathogen-rich aerosol, showing almost ~6 log reduction in the bacterial count [Fig. 3(a)].

Bactericidal kinetics of coated surfaces

There is a need for surfaces to rapidly kill pathogens (e.g. bacteria, fungi, viruses) upon exposure. If observed, such expeditious bactericidal effect can enable QAP coated surfaces to prevent the onset of transmission upon touch by healthy individuals. We therefore examined the temporal kinetics of bactericidal effect for QAP coated nylon surfaces. Bacterial suspension ($\sim 10^6$ CFU/mL; 10 μ L) was dropped on the surfaces followed by incubation at 37 °C. At time points 0 min, 20 min and 45 min, surfaces were dragged on nutrient agar plates. Uncoated surfaces showed survival of bacteria, with almost 4 log (CFU) bacterial cells being present even at 45 min. On the other hand, in case of QAP coated nylon surfaces, 3.5 log (CFU) bacteria could be seen at 20 min [Fig. 4(b)]. However, at 45 min, a rapid lowering in bacterial count could be

Figure 3. (a) Antibacterial activity of QAP coated surfaces upon spraying of plasma suspension of bacterial cells. (b) Bactericidal kinetics of QAP coated nylon surfaces against MRSA R3545. (c) Antibacterial adherence of QAP coated surfaces against MRSA R3545. (d) Anti-fungal activity of QAP coated surfaces against *C. albicans* spp. Arrow shows the direction dragging. (*) indicates bacterial count < 50 CFU/mL.

Figure 4. Virucidal activity against A/NWS/33 (H1N1) of QAP coated PVC surface as observed using plaque assay. (a) Uncoated PVC surface and (b) QAP coated PVC surface.

observed, suggesting ~ 3.9 log reduction in the bacterial count. This result showed the fast bactericidal effect exerted by QAP coating [Fig. 3(b)].

Reduction in bacterial adherence by the coated surfaces

A major threat posed by pathogenic bacteria is their adherence on different surfaces leading to formation of biofilms, in which bacterial cells remain shielded in an exopolysaccharide matrix, thereby rendering antibiotics ineffective for use.^[26] Almost 50% of surface mediated infections are biofilm associated. Therefore, it is prudent to investigate the potency of QAP coated surfaces to prevent the adherence of bacterial cells thereby inhibiting the possibility of biofilm formation. QAP coated PVC and aluminium surfaces were incubated with MRSA under static conditions for 4 days. In case of uncoated PVC surfaces, upon incubation for 4 days, a biofilm growth consisting almost 6.5 log (CFU/mL) bacteria could be observed. However, when the PVC surfaces were coated QAP, the bacterial count on the surface was lowered by 3 logs. In case of QAP coated aluminium surfaces, ~ 3.2 log lowering was observed in the bacterial count on the surface compared to that of bare aluminium. Therefore, QAP coating led to the lowering of bacterial adhesion and hence, nullifying the possibility of biofilm formation on both metallic and non-metallic surfaces [Fig. 3(c)]. Overall, the contact active bactericidal nature of the polymeric coating is believed to inhibit the adherence of bacterial cells diminishing their colonization ability and biofilm formation.

Antifungal activity of coated surfaces

There has been an increasing trend in the incidence of fungal infections in the nosocomial settings. It is predicted that the rate of nosocomial fungal infections will continue to rise in frequency in the coming decades. One of the predominant nosocomial fungal pathogens are *Candida* spp.^[27] The difficulty to diagnose such infections coupled with the growing resistance towards existing antifungal therapy lead to high morbidity and mortality. Therefore, antifungal activity of the QAP coated surfaces was tested against fluconazole-resistant strains of *C. albicans*. All the uncoated surfaces showed thick fungal lawn indicating the presence of large number of live fungal cells [Fig. 3(d)]. However, the QAP coated surfaces did not display any fungal growth proving their superior antifungal potency effecting 100% killing of fungi cells [Fig. 3(d)].

Antiviral activity of the coated surfaces against influenza virus

Every year, global population evidences a significant burden of hospitalization and morbidity owing to influenza viruses.^[28] With the onset of new strains of the virus, such as H1N1, it has

been imminent than ever to prevent their transmission. In the current situation of coronavirus pandemic, we have once again realized the role of commonly handled objects in the spread of viral diseases. Therefore, QAP coated PVC surfaces were investigated for their efficacy to kill influenza virus. In case of uncoated surfaces, 1.60×10^4 PFU were observed which was not significantly different from the initial count of 2.40×10^4 PFU. However, in case of QAP coated PVC surface, no plaques were observed, proving the efficacy of the coating to reduce the influenza burden by killing almost $\sim 10,000$ viruses (Fig. 4). The hydrophobic quaternary polymeric coatings are believed to have an edge over other amines as they can interact with lipid-laden membranes of microbes thereby lysing and inactivating them.

Conclusion

In conclusion, we have demonstrated the development of a quaternary antimicrobial paint (QAP) based on polyethyleneimine (PEI) which can be coated on different surfaces in a simple drop-casting method. The simplicity of the coating fabrication technique eliminates the requirement of sophisticated instruments or skilled personnel. The coated surfaces showed complete killing of drug-resistant bacteria and fungi. Moreover, the coated surfaces showed killing of $\sim 10,000$ human influenza virus cells (H1N1). All the results suggest that QAP holds great promise to be developed as an effective coating formulation for different high-touch surfaces, such as personal protective equipments, tables, desks, medical instruments to tackle the threat of microbial infections.

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Declarations

Conflict of interest

The authors declare no competing conflict of interest.

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