Materials Today Advances 19 (2023) 100398

Contents lists available at ScienceDirect

Materials Today Advances

journal homepage: www.journals.elsevier.com/materials-today-advances/

Water activated catechol adhesive allows dip coated antimicrobial coatings

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ARTICLE INFO

Article history:
Received 14 March 2023
Received in revised form
15 June 2023
Accepted 20 June 2023
Available online 29 June 2023

Keywords:
Catechol adhesive
Water activation
Lap shear adhesion
Rheology
Antimicrobial coating

ABSTRACT

Bioadhesives comprising of catechol crosslinkers have displayed broad utility against both soft and hard substrates. However, catechol's two-part adhesion chemistry requires oxidative chemicals that are detrimental to organic substrates. Herein, a water-activated adhesive with inherent antibacterial properties is prepared by grafting catechol groups onto branched polyethylenimine (PEI-DBA_{20}). The resultant PEI-DBA_{20} is stable in organic solvents but undergoes curing in the presence of water. The in-built oxidation method relies on the close proximity of catechol/Schiff base functional groups that form tautomers in the presence of aqueous solvents. The curing mechanism is demonstrated by dip coating hydrated substrates, where the grafted dendrimers subsequently crosslink and form thin films. Coated PET films and polyester textiles exhibit an antimicrobial surface with 4–6 log reduction against model Gram-negative bacteria.

1. Introduction

Marine mussels are noted for their robust adherence to rigid substrates in both fresh and salt-water environments. This is possible with specialized foot proteins rich in catechols that are oxidized to quinones [1,2]. Quinones serves as a reactive crosslinker which can molecularly interact with a variety of materials including rocks, metals, and plastics [3–5].

Design of synthetic macromolecular adhesives exploit these observations by grafting quinone precursors on hyaluronic acid [6–8], chitosan [9–11] and other natural polymers. Most initiation methods rely on oxidation reagent mixing ($NaIO_4$, H_2O_2) [6–8,12,13] to transform catechol into quinone. Alternative approaches are evaluating electrochemical oxidation to avoid 2-part reagent mixing and implement one component (1C) formulation [14]. This strategy requires a voltage stimulus to trigger the donor-acceptor redox pair, creating quinones. A serendipitous observation found water-based solutions would initiate spontaneous curing after an initial lag period of 20 min. The water-activated adhesives may have utility as water-based coatings and adhesives, but need improvements in material properties, including reducing the gelation time (e.g., <10 min) while having sufficient shelf stability in organic solvents (>1 month). The oxidation of catechol to quinone is known to be pH dependent where alkaline pH (>8.5) accelerates the rate of catechol oxidation [3,15]. Thus, it was hypothesized that a dendrimer with a higher density of basic amines may exhibit a higher kinetic conversion to quinone and yielding a shorter gelation time. For the first time, catechol acceptor-donor pairs are grafted on branched polyethylenimie (PEI-DBA_{20}). PEI has a higher amine density and much cheaper compared to PAMAM dendrimers. PEI's natural biotoxicity coupled to a water activated adhesion should allow antimicrobial coatings through a simple dip coating process.

For the first time, we report the beneficial attributes of adhesion strength, and antimicrobial activity of Schiff base grafted catechols on PEI dendrimer (PEI-DBA_{20}). The advantages over previous formulations include accelerated curing, adherence to plastics and textiles, and a demonstration of aqueous-based formulations that yield antibacterial coatings.

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https://doi.org/10.1016/j.mtadv.2023.100398

2. Experimental

2.1. Materials

Polyethylenimine (PEI), 3,4-dihydroxybenzaldehyde (DBA), phosphate buffer, anhydrous methanol, absolute EtOH, diethyl ether (Et_2O; inhibitor free, >99.99%), were purchased from Sigma-Aldrich, Singapore. The disposable 3-electrode chips were purchased from Zensor R&D Company, Taipei, Taiwan. Dehydrated Luria-Bertani (LB) culture media and dehydrated LB-Agar was purchased from BD Difco™.

2.2. Synthesis procedure

Among three types of amines present in 28 kDa PEI, an estimate of 194 primary amines are available for Schiff base grafting calculation shown on SI page 3. For comparison to G5-DBA, 20% of primary amines 39:1 DBA:PEI mol ratio is sought.

The synthesis scheme is shown in Fig. S1. Nitrogen was bubbled through a solution of PEI (3.33 g, 0.133 mmol, 1.0 equiv) in anhydrous MeOH (20 mL) for 10 min. A solution of DBA (714 mg, 5.16 mmol) in anhydrous MeOH (5 mL) was added dropwise over a period of 5 min. The reaction mixture was stirred overnight at room temperature. It was poured in a large volume of deoxygenated anhydrous Et_2O (100 mL) and stirred vigorously for 30 min. The resulting precipitation was dried *in vacuo* to give PEI-DBA_{20} as a viscous brown liquid in quantitative yield. The product PEI-DBA_{20} is characterized by ^{1}H NMR and UV−vis spectroscopy. ^{1}H NMR (400 MHz, CD_3OD) δ 8.41−7.79 (brs, 38.8H), 7.31−6.56 (m, 116.4H), 3.90−3.53 (m, 77.6H), 3.07−2.22 (m, 2246.4H); UV−vis (PBS buffer), λ_{max} (nm) 346, 410−540.

2.3. pH measurement

The pH of PEI and G5-PAMAM at three different concentrations in water (0.1 mM, 0.5 mM, and 1.0 mM) are measured with Thermoscientific ORION STAR A211 pH meter at 24 °C. Before measuring pH of samples, the equipment is calibrated with three different buffer solutions (pH 4.0, 7.0, and 10.0) as instructed in the pH meter manual.

2.4. Cyclic Voltammetry (CV)

Voltammetric measurements were performed using a Metrohm Autolab PGSTAT302 N potentiostat in a three-electrode setup. A 3 mm diameter planar glassy carbon disk (Metrohm) was used as a working electrode in conjunction with a platinum plate counter electrode (Metrohm) and Ag/AgCl reference electrode (filled with 3.0 M KCl solution). The cyclic voltammetric data were recorded in phosphate buffer solution (0.13 mM), at the scan rate 0.1 V/s. All voltammetric experiments were conducted under argon atmosphere, at room temperature in a Faraday cage. Before recording each voltammogram, the working electrode was cleaned by polishing with alumina oxide (grain size 0.3 μm) slurry on a Buehler Ultra-pad polishing cloth, rinsing with ethanol, and then drying with a lint-free tissue.

2.5. Preparation of PEI-DBA_{20} adhesive formulation

The 30 wt% formulation was prepared by adding 60 mg PEI-DBA_{20} in 140 μL PBS buffer (pH ~ 7.2) and vortexing the mixture until a clear homogeneous solution is observed. A sample of 20 μL was loaded onto the 3-electrode (Zensor) plate for rheology measurements.

2.6. Rheology measurements

Dynamic rheology and electrorheology (−1.0 V, −2.0 V, −5.0 V) measurements of PEI-DBA_{20} adhesive were performed on a rotational/oscillating rheometer (MCR102, Anton Paar, Singapore) coupled with a portable potentiostat (Vertex, Ivium Technologies, Netherlands) connected to an immobilized disposable 3-electrode polypropylene-based Zensor® chip. The Zensor chip is embedded with a 3-mm diameter glassy carbon as a working electrode (WE), glassy carbon as the counter electrode (CE), and an Ag/AgCl pellet as the reference electrode (RE). An electrically insulated ceramic rheometer probe with 10-mm diameter parallel-plate geometry (PP10 Ceramic) serves as the measuring probe and directly interfaces to the 3 mm diameter WE in contact with 20 mg of PEI-DBA_{20} adhesive formulation at 0.3 mm probe/plate gap. Complex modulus had a data acquisition rate of 1 Hz using a 10 Hz angular frequency and 10% strain amplitude. The potentiostat applied programmed voltages (−1.0 V, −3.0 V, −5.0 V) to the PEI-DBA_{20} adhesive formulation. All samples are evaluated in triplicate with minimum torque requirement of 20 nN m, which is 4x instrument limit of detection.

2.7. Lap shear adhesion test

Lap shear adhesion studies were performed according to the ASTM standard F2255-05. Shear adhesive strength failure was measured using a tensile tester (Chatillon Force Measurement Products, USA) using a 50 N loading cell and at a linear rate of 3 mm min^{-1}. PEI-DBA_{20} adhesive formulation (50 μL) was applied on 2 x 2 cm^2 area of the substrate (collagen, glass slide, PMMA slide), which was then mounted onto the other substrate of the same type and secured using paper clips. In case of collagen film substrates, PEI-DBA_{20} adhesive sandwiched by two hydrated collagen films (collagen/PEI-DBA_{20}/collagen sandwich structure), was placed in between two glass slides and fastened with paper clips. The adhesive was allowed to cure for 30 min at ambient conditions before lap shear adhesion study performed. Thus, during the curing process the scope for water evaporation is minimal.

2.8. Determination of minimum inhibitory concentration and minimum bactericidal concentration

Gram-negative *Escherichia coli (E. coli)* strain BL21 (DE3) was cultured to a mid-log phase in the Luria-Bertani (LB) liquid broth media at 37 °C according to ATCC protocols and diluted to 2×10^5 colony forming units (CFU) mL^{-1} in LB media. Stock solutions of PEI-DBA_{20} were prepared in PBS (pH 7.4) at a concentration of 16 mg mL^{-1}. They were serially diluted 2-fold in PBS (0.125−16 mg/mL) and 100 μL of each dilution was placed in a 96-well plate (Greiner Bio-one, Germany). Then, 100 μL of bacterial suspension was added to each well with PEI-DBA_{20} solution. The plate was incubated at 37 °C overnight and observed by the naked eye. Bacterial growth made the suspension appear cloudy, while the suspension with no bacterial growth remained clear. Subsequently, 5 μL of the suspension from the wells were diluted 1-10^7 times and 5 μL of each dilution was placed on LB agar and incubated at 37 °C overnight to observe the viability of the bacteria and calculate the colony forming units/mL (CFUs/mL). The dilution with colony forming units (CFUs) ranging from 5 to 20 were employed. The lowest concentration of the compound that inhibited the growth of bacteria was recorded, the minimum inhibitory concentration (MIC). The lowest concentration of the compound that killed 99.9% of the initially inoculated bacteria was recorded, the minimum bactericidal concentration (MBC).

2.9. Coating of PET sheets/polyester cloth fibres with PEI-DBA_{20}

Polyester terephthalate (PET) sheets (20 μm thick) and polyester cloth fibres (CF) were kindly donated by Sportmaster, Singapore. Samples were cut into 2 x 2 cm^2 pieces and dipped in PEI-DBA_{20} dissolved in in anhydrous ethanol at concentration of 1 and 10% w/v. Three dip coatings of PET sheets and polyester cloths were performed for 1% solution, and one for 10% PEI-DBA_{20} solution. The substrates were left to dry for 24 h at room temperature in a humidified atmosphere of 60–80%.

2.10. Evaluation of antimicrobial property of coated substrates

Gram-negative *Escherichia coli* (*E. coli*) strain BL21 (DE3) was cultured to a mid-log phase in the Luria-Bertani (LB) liquid broth media at 37 °C according to ATCC protocols. Briefly, one loopful of bacterial culture was inoculated in 10 mL of LB broth in an incubator shaker (New Brunswich Scientific Excella E24) at 37 °C at 200 rpm overnight. The bacteria were revived by inoculating 100 μL of the bacterial suspension in 10 mL of fresh broth under the same incubating conditions for 16 h. The bacterial suspension was centrifuged at 4000 x G for 10 min at room temperature to harvest bacterial cells. The supernatant was removed, and the bacterial pellet was resuspended in 10 mL of deionized water using a vortex mixer (Vortex Genie 2). The washing process was repeated thrice. Pellet was dispersed in 2 mL of PBS to get the bacterial suspension. Neat PET sheets, polyester CF, and respective coated (PEI-DBA_{20}) substrates were placed into wells of 12-well tissue culture plate sterile (TCPS) (triplicate). Five drops comprising of 10 μL of bacterial suspension was dropped directly onto the substrates. For control, drops were placed directly on the TCPS. The outer space of the well plates were filled with PBS to minimize evaporation of the droplets. The plate was incubated at room temperature for three time durations of 6, 24, and 48 h. Each well was extracted with 1 mL PBS. Substrates were then placed in 1.5 mL tubes with 1 mL PBS and vortexed for 1 min to detach bacterial cells. The bacterial suspension collected from substrates and those vortexed were then diluted and grown on LB-agar plate for CFUs/mL counting evident by the presence of droplets after 24, 48 h of study (Figs. S10 and S11). In case of cloth fibre and PET sheets coated with PEI-DBA_{20}, the droplets are not visible as they spread out on the relatively hydrophilic surface due to presence of PEI (further supported by contact angle Fig. S9). Visible droplets in case of controls thus confirm little to no evaporation which might significantly impact the results of antibacterial study.

3. Results

The targeted one-component (1C) adhesive (PEI-DBA_{20}) is synthesized in a one-step synthetic procedure. Shelf stability was evaluated for two formulations: 1) *neat* adhesive at −20 °C under inert nitrogen or 2) alcoholic solution (1.68 mM) under ambient conditions. The curing reaction of PEI-DBA_{20} in PBS buffer solution is triggered by water and/or oxygen. Hypothesized base accelerated reaction of PEI-DBA_{20} is shown in Fig. 1a. Rheological investigations on 3-electrode Zensor chip evaluate the shear rheology of PEI-DBA_{20} under ambient or cathodic activation. The latter evaluates if the redox chemistry can be accelerated or retarded through electric fields, as shown previously with PAMAM dendrimers [14]. Lap shear measurements evaluate its maximum adhesive strength and mode of failure on both rigid surfaces of glass/PMMA and soft collagen substrates. For antimicrobial coating investigations the substrates are dip coated in ethanolic (absolute) solution of PEI-DBA_{20}. The volatile/hygroscopic nature of EtOH and PEI renders a thin coating of PEI-DBA that is immediately hydrated from humidified laboratory atmosphere (relative humidity of 60–80%). The dual mechanism of rapid solute concentration (by EtOH evaporation) and hygroscopic moisture attraction (by PEI) serve to initiate water-activated crosslinking and polymerization. The crosslinked cationic coating is then evaluated for antimicrobial properties on dip coated polyester substrates.

3.1. PEI-DBA_{20} characterization

Previously it was found that grafting of 20% DBA grafted onto a 5th generation PAMAM dendrimer (G5-DBA_{20}) had the optimal solubility and viscoelastic material properties. E.g. viscous (G" > G′) before curing and soft elastic (G' > G") after curing [14]. This served as the starting point for 20% grafting of the surface 1° amines of PEI with DBA, which is calculated by ^{1}H NMR and UV–vis spectroscopy (see SI). The three catechol aromatic protons (b, c, d) are assigned to peaks at 6.5–7.6 ppm and used for quantitative grafting evaluation [14]. The imine (CH═N) peak is assigned to the peak at 8.2 ppm. The aliphatic CH_2 protons of PEI is assigned to 3.5–3.9 and 2–3 ppm respectively. The calculation from ^{1}H NMR peaks integration shows that the % of grafting of DBA in PEI-DBA_{20} is 20 ± 1% (see SI).

The Schiff base grafting of PEI-DBA_{20} is further confirmed by UV–vis absorption spectra in PBS buffer solution (10^{-6} M), which exhibits several fold increases in the intensity of catechol absorption peak (346 nm) in comparison to 3,4-DBA and formation of broad peaks at (410–540) nm due to the formation of quinone and subsequent polymerization (Fig. S2).

3.2. Elastic modulus of PEI-DBA_{20} adhesive increases with time

Oscillatory rheological measurements investigate the viscoelastic properties of PEI-DBA_{20} adhesive before, during, and after curing. Fig. 2a shows the probe and base within the rheological study. Note that the sample is electrically isolated by the inert (non-conductive) ceramic probe, whereas metallic probes can induce catechol metal-cation chelation [16,17]. PEI-DBA_{20} adhesive is diluted in PBS buffer and immediately loaded (20 μL) on a Zensor chip electrode fastened to the bottom plate of the rheometer. PEI-DBA_{20} at 30 wt% in aqueous buffer is the optimum concentration to yield the highest shear modulus (Fig. S3). All the subsequent rheological and lap shear investigations are performed at this concentration. Steady state rotational viscosity measurement of PEI-DBA_{20} formulation is performed at the fixed shear rate of 62.8 rad s^{-1} (10 s^{-1} @ 3/4 R) to break any reversible intermolecular attractive forces. No deviations in apparent viscosity (1821–3367 mPa s) after shear suggests that the formulation is stable during the first 30 s of shearing, as shown in Fig. 2b. Dynamic shifts in G' & G″ are then monitored as the formulations crosslink and transition from a liquid to an elastic hydrogel. Curing under ambient conditions is attributed to the auto redox reaction in which tautomers are formed between catechol and the Schiff base that eventually leads to formation of quinone crosslinker in the presence of water. Complex quinone mediated crosslinking and other redox reactions then ensues between the PEI surface amines via Michael addition, ketimine formation, or combination thereof [18–20]. Fig. 2c displays that, initially (up to 4 min) the formulation exhibits viscous behaviour with loss modulus (G″) exceeding storage modulus (G′). The adhesive attains gelation at 4 min, defined as the time point when G′ exceeds G". Storage modulus (G′) keeps increasing up to the 30 min evaluation period and is expected to continue for several hours due to crosslink density and matrix dehydration. At 30 min, the tack strength is measured to be 3.0 kPa (±0.6 kPa). A prolonged curing investigation (Fig. 2d) shows that 90 min is required to hold a 1 kg. f per square cm (9.8 kPa).

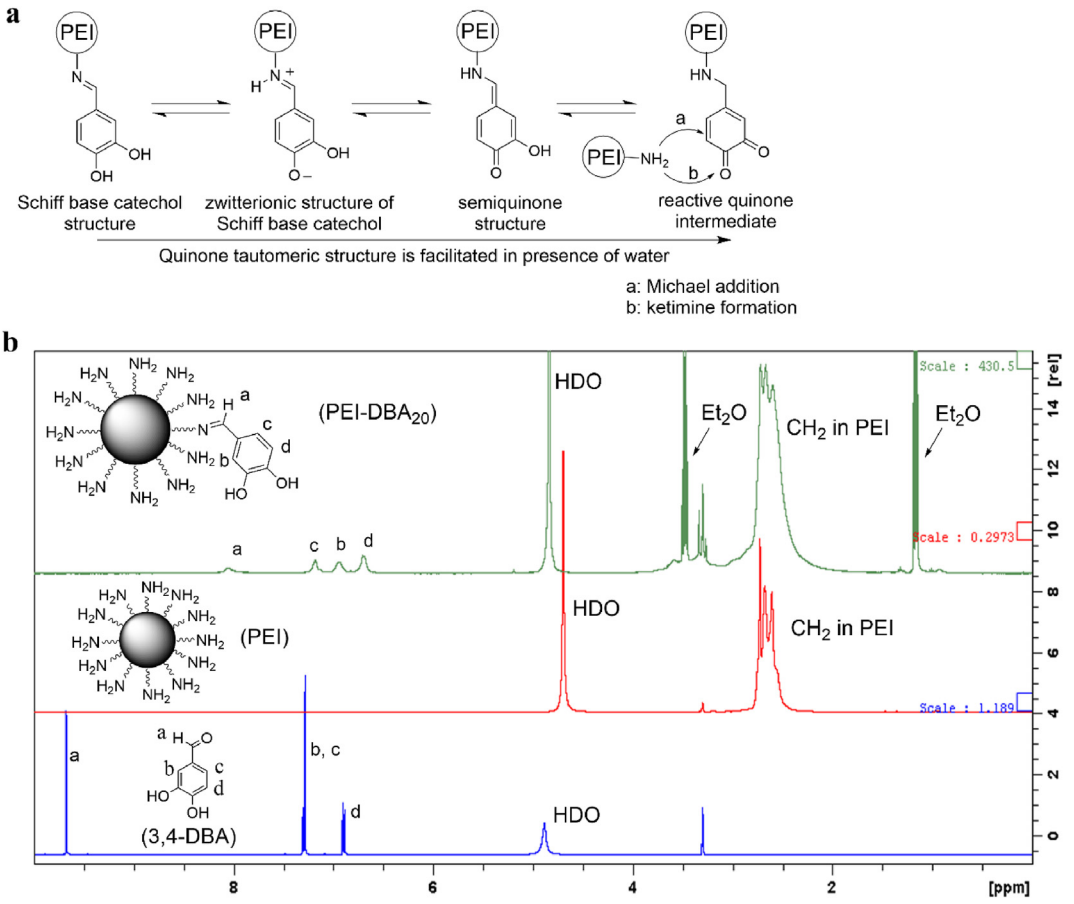

Fig. 1. (a) Tautomeric structures for the formation of reactive quinone from catechol/Shiff base pair in presence of water. (b) ^{1}H NMR spectra of 3,4-DBA, PEI and PEI-DBA$_{20}$ in CD_3OD.

Thereafter, logarithmic increases in storage modulus (G') is observed. At the end of 2.5 h curing, the final measurement of 14.8 kPa (±1.8 kPa) is recorded.

3.3. Applied electric field has no impact on gelation time

Previous investigations on catechol grafted PAMAM-G5 adhesive (G5-DBA$_{20}$) determined cathodic potential reduced gelation time and accelerated the complex modulus [14]. Cyclic voltammogram of PEI-DBA$_{20}$ exhibits low value of oxidation potential (E_{ox1} = 0.31 V Vs Ag/AgCl) for catechol oxidation (Fig. S4). We hypothesized that PEI-DBA$_{20}$ would display similar acceleration in gelation time and shear modulus. Accordingly, PEI-DBA$_{20}$ adhesive is voltage stimulated for a period of 30 min and its real time rheological behaviour is studied. Fig. S5a and Fig. S5b show that there is no correlation of complex modulus or gelation time of PEI-DBA$_{20}$ with respect to voltage. This suggests that there is little to no external influence on the redox chemistry from external stimuli.

3.4. Cured material exhibits linear viscoelastic regime up to 50% applied strain

The elastic behaviour of the hydrogel at the end of 30 min of water exposure is investigated by a series of increasing shear strains, also known as amplitude-sweep test. Fig. 3a displays that there exists a linear viscoelastic regime (LVR) of both G' and G" throughout the range of up to 50% strain. This indicates that the cured material effectively is elastic under the limited 50% strain. Beyond the LVR, the viscoelastic material displays evidence of strain hardening at 80–100% strain before yielding, fracture, or a combination thereof.

3.5. Lap shear adhesion strength is highest on collagen and lowest on glass

Adhesive performance on complex substrates is evaluated through a lap shear adhesion. Fig. 3b illustrates the experimental setup. The adhesion strength on three different substrates - collagen, PMMA, and glass is 35.5 kPa (±1.9 kPa), 34.1 kPa (±6.4 kPa), and 5.9 kPa (±0.3 kPa) respectively (Fig. 3c). Authors speculate a correlation on absorbed surface water, but several other criteria could also be responsible for low adhesion strength of glass, including surface roughness, oxygen permeability, and inert functional groups.

3.6. PEI-DBA$_{20}$ exhibits antimicrobial activity against gram negative E.coli

The antimicrobial activity of PEI-DBA$_{20}$ towards Gram-negative (*Escherichia coli*) bacteria is investigated using the microplate dilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). PEI-DBA$_{20}$ serial diluted in PBS (Fig. 4a) and subsequently incubated with *E. coli* in LB media at 37 °C overnight. Fig. 4b depicts PEI-DBA$_{20}$ with concentration 0.125 mg/mL has the lowest concentration that inhibits the growth as compared to control. Concentrations higher

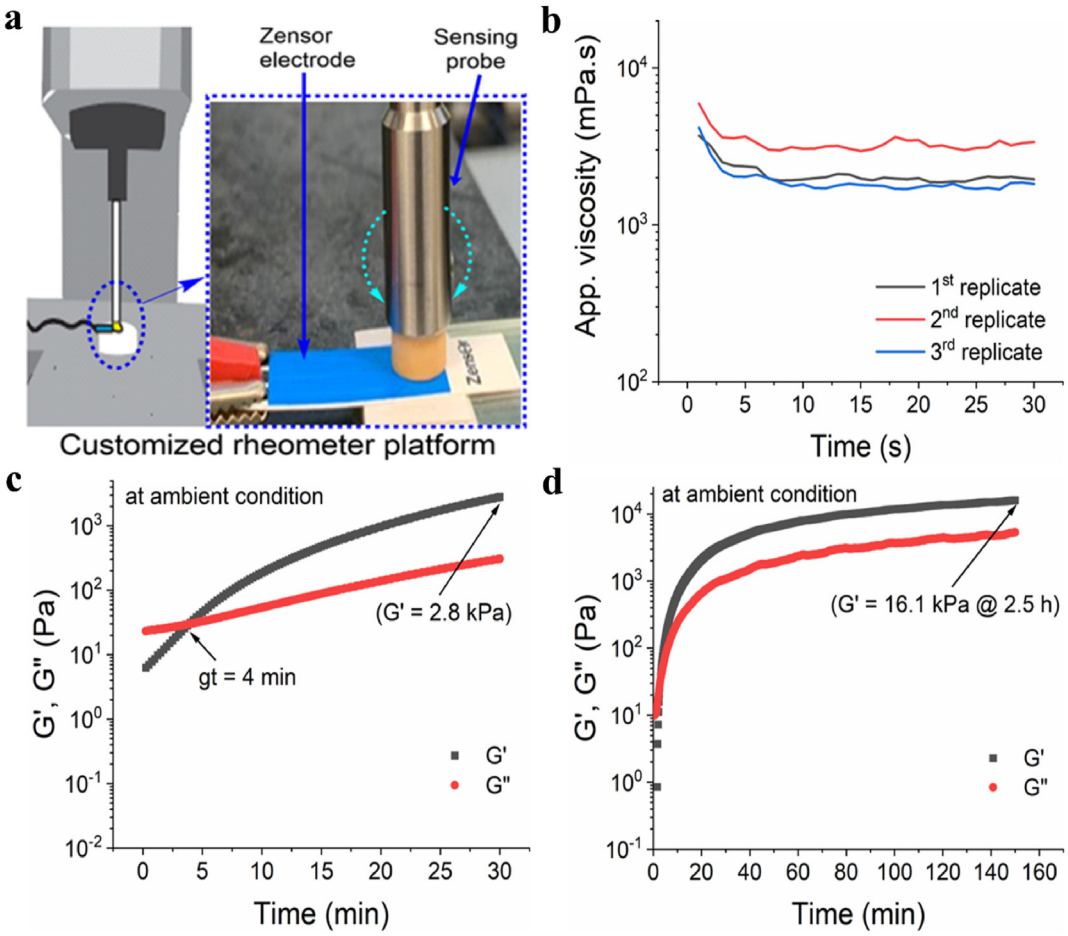

Fig. 2. (a) Experimental set-up for real time rheology of PEI-DBA_{20}. (b) Apparent viscosity of PEI-DBA_{20} adhesive at fixed shear rate of 10 s^{-1}/62.8 rad s^{-1}. (c) 30 min curing of PEI-DBA_{20} adhesive at ambient condition (0.0 V). (d) 2.5 h curing of PEI-DBA_{20} adhesive at ambient condition (0.0 V).

than 0.125 mg/mL further reduce the number of viable bacteria upon overnight incubation. PEI-DBA_{20} concentration of ≥2 mg/mL exhibits bactericidal activity.

3.7. *PEI-DBA_{20} coated substrates exhibit >6 log reduction of E.coli*

Presence of cationic primary amine (-NH_2) in the PEI backbone provides inherent antimicrobial activity (Fig. 4). Thus, it was hypothesized that substrates coated with water activated PEI-DBA_{20} will exhibit antimicrobial property if the films remain adherent. This is challenged by exposing the coated substrates with Gram negative bacteria. Two coated substrates i.e., PET sheets (PET) and polyester cloth fibre (CF) are prepared by dip-coating with two concentrations of PEI-DBA_{20} solution (1% or 10% w/v in anhydrous ethanol) (Fig. 4c). The coated substrates were evaluated for variations in color, weight per unit area and contact angle (hydrophilicity) (SI, Fig. S9). Antimicrobial activity of substrates coated with PEI-DBA_{20} is determined using the method illustrated in Fig. 5a. Bacterial suspension are placed on the coated and uncoated substrates and incubated up to 48 h. For the initial experiment all the substrates i.e. tissue culture plate sterile (TCPS, control), uncoated PET (control), PET_1% and PET_10% are incubated with bacterial suspension drops for 6 h (Fig. 5b). The coated substrates are evaluated in two forms: 1) dry form (D), 2) wash with PBS for 15 min to remove unbound PEI-DBA_{20} (W). TCPS and PET controls exhibit 10^7 CFU/mL, wherein PET_1% (coated with 1% PEI-DBA_{20}) exhibits 1 log reduction (10^6 CFU/mL) in dry form (D) and no reduction was observed for washed PET_1% (W). On the other hand, PET_10% (coated with 10% PEI-DBA_{20}) exhibited 4 log reduction (10^3 CFU/mL) in dry form (D) and >6 log reduction (<10 CFU/mL) in washed form (W). PET_1% substrates are then evaluated with extended incubation time of 24 and 48 h. TCPS and PET control have no changes in bacterial cell viability after 24 h (Figs. 5c) and 48 h (Fig. 5d) incubation. PEI_1% on the other hand exhibited >6 log reduction (<10 CFU/mL) in both dry and washed form (W). The increased antimicrobial activity can be attributed to several events; evaporation, increased cationic surface exposure or combination thereof. CF_1% (coated with 10% PEI-DBA_{20}) exhibits 2 log reduction (10^6 CFU/mL) in dry form (D) and washed form (W). On the other hand, CF_10% (coated with 10% PEI-DBA_{20}) exhibited >6 log reduction (<10 CFU/mL) in both dry form (D) and washed form (W). CF_1% has a ≥4 log reduction upon ≥24 h (Fig. 5e, f, g).

Bacterial suspension droplets are visible in case of TCPS and PET controls after 24, 48 h of study confirming there is little to no evaporation (Figs. S10 and S11). In case of cloth fibre and PET sheets coated with PEI-DBA_{20}, the droplets are not visible as they spread out on the relatively hydrophilic surface due to presence of PEI (further supported by contact angle Fig. S9).

4. Discussion

Existing catechol adhesives require an external trigger (e.g. toxic chemicals, transition metal ions or a voltage source) to initiate bonding through covalent linkage or via the formation of

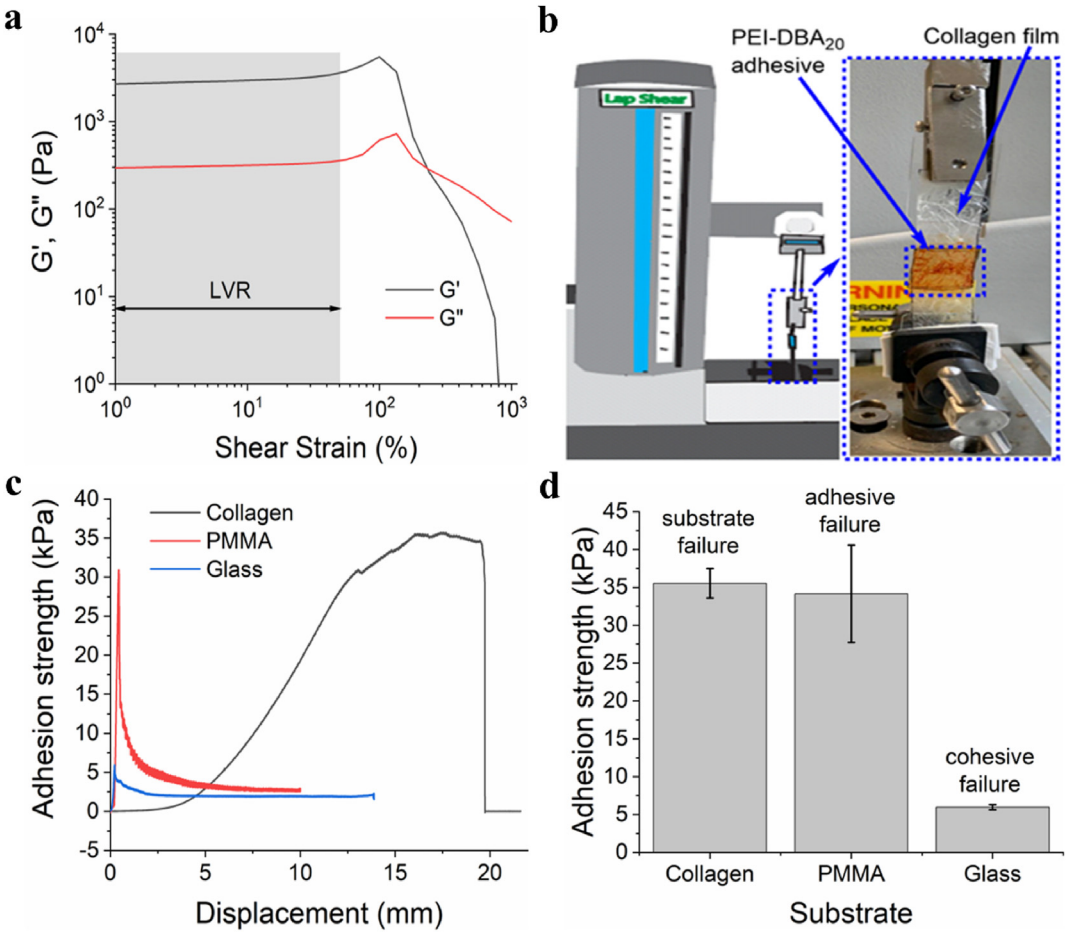

Fig. 3. (a) Linear viscoelastic region (LVR) of PEI-DBA_{20} at shear strain from 1 to 1000%. (b) Experimental set-up for lap shear adhesion test. (c) Representative graphs of lap shear adhesion test of PEI-DBA_{20} on collagen, PMMA, and glass substrates. (d) Bar graph for the lap shear adhesion strength of PEI-DBA_{20} on collagen, PMMA and glass surfaces.

coordination complex. Synthesis of most of the catechol adhesives involves cumbersome protection/deprotection procedure or extended reaction time [21–23]. In addition, most of the catechol adhesives have not been designed to possess antibacterial activity.

Herein PEI-DBA_{20} is prepared by employing a one step, scalable synthesis strategy. The combination of a dendrimer with higher basicity branched PEI and the presence of moisture shifts the tautomer equilibrium into a spontaneous auto redox mechanism. Where the catechol/Schiff base pair is stable in anhydrous alcohols, catechol converts to quinone intermediates when diluted in water. Subsequent crosslinking between the nucleophilic amine groups and quinones then ensues. Adhesion is demonstrated on both flexible and rigid substrates (35.5 kPa ±1.9 kPa on collagen, 34.1 kPa ±6.4 kPa on PMMA, and 5.9 kPa ±0.3 kPa on glass). Thus, PEI-DBA_{20} overcomes the 2-part limitation inherent in most catechol grafted polymer adhesives. The positively charged amines of PEI-DBA_{20} render it with antibacterial activity against model bacteria *E. coli* at concentrations >0.125 mg/mL. Permeable and nonporous substrates coated with PEI-DBA_{20} also exhibit reduction in bacterial cell viability (2–6 log reduction) compared to no reduction in case of neat substrates. The water activated adhesion, rapid gelation, and inherent antibacterial property makes PEI-DBA_{20} a promising material for coatings on a wide range of substrates.

PEI-DBA_{20} adhesive is made up of two components - catechol functional group and the branched PEI dendrimer. The PEI dendrimer alone is highly miscible in both organic and aqueous phases and unlikely to covalently insert surfaces such as glass, plastic, or fabric. Therefore, PEI has been used in combination with other crosslinking agents to develop adhesive or coating material for chelation [24], electrostatic interaction [25], or crosslinking polymerization [26]. PEI's cationic nature is destructive for negatively charged cell membranes, leading to cell depolarization, membrane disruption and cell lysis [27]. By grafting catechol tautomers onto branched PEI, the antibacterial properties could be immobilized in straightforward manner by simple dip coating. PEI's basic nature was found to accelerate the curing mechanism of catechol/Schiff base tautomers—curing of PEI formulations was observed in <5 min, whereas previous PAMAM formulations ranged from 20 to 30 min. The self oxidation of catechol is known to be accelerated at higher pH [3,14,15,28]. Indeed, even PAMAM dendrimer (G5-DBA_{20}) was found to have accelerated curing when tertiary amines were added (Fig. S6). Aqueous solution of PEI at three different concentrations showed much higher pH than that of G5-PAMAM (Table S1).

The PEI-DBA_{20} adhesive was synthesized in one step process by stirring a mixture of PEI and DBA in MeOH. Neat adhesive can be purified by diethyl ether precipitation to provide PEI-DBA_{20} as a viscous brownish gum (Fig. S1). In this method, it can be stored and redissolved in anhydrous solvents.

The rheological investigation of PEI-DBA_{20} found a moderate value of apparent viscosity (1.8–3.4 Pa s, similar to honey) suggests that little to no polymer entanglement is present under the 30% solute loading. The 5 min gelation time offers a balance between

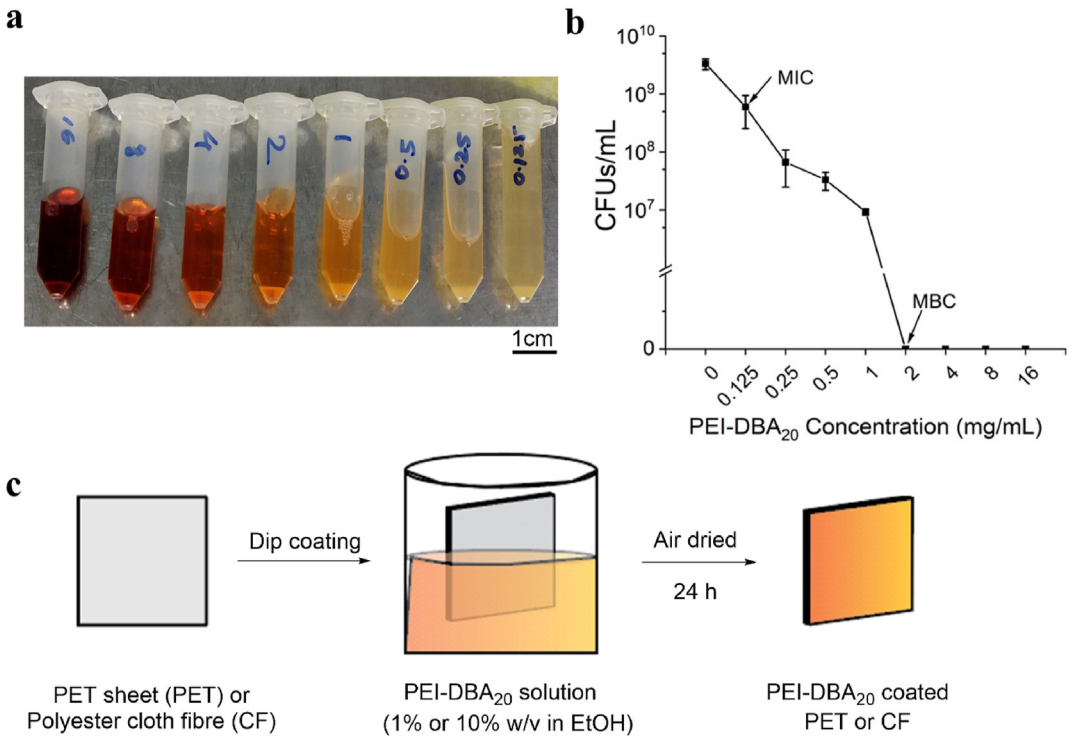

Fig. 4. (a) Serial dilutions (2-fold) of PEI-DBA_{20} in LB-Agar ranging from 0.125 to 16 mg/mL employed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). (b) MIC and MBC of PEI-DBA_{20} determined using dilution/agar test evaluated against gram-negative *E. coli*. (c) Schematic representation of dip coating procedure of PET sheet (PET) and PET cloth fibre (CF) with PEI-DBA_{20} solution, later employed to evaluate antimicrobial activity.

enough time for application, yet rapid gelation time for adhesion and coating applications. When diluted, the appearance of a broad peak at 480–520 nm shows a steady increase in UV–vis absorption, attributed to quinone formation and polymerization (Fig. S2) [29,30]. The rheological investigation exhibits that the 30 wt% PEI-DBA_{20} formulation displays a gelation period of 5 min, with a storage modulus of 2.8 kPa after 30 min (Fig. 2c). Such a low G' suggests this would be a soft and flexible coating material that could conform to most surfaces. The crosslinking polymerization continues at ambient condition and at the end of 2.5 h the value of storage modulus is found to be 16.1 kPa. The crosslinking of PEI-DBA_{20} is believed to occur via the auto redox reaction in which catechol is oxidized to quinone by donating $2e^-$ and $2H^+$ (donor) and simultaneously the Schiff base is reduced to amine by accepting the $2e^-$ and $2H^+$ (acceptor) (Fig. 1). Voltage application was therefore speculated to enhance crosslinking kinetics by accelerating the auto redox process. Voltage application has minimum effect on modulus or gelation time of PEI-DBA_{20} adhesive. It can be inferred from this observation that PEI-DBA_{20} is more stable than G5-PAMAM and relatively stable in absence of moisture. High degree of basicity of PEI-DBA_{20} dendrimer assists the catechol O–H deprotonation, setting up a metastable intermediate in organic solvents, but shifts to self-redox processes in moisture. The highly reactive quinone subsequently undergoes Michael addition reaction with various nucleophiles available in the adhesive matrix (cohesion force) as well as on the substrate surface (adhesion force). Adhesive property is largely due to the step growth crosslinking polymerization of PEI-DBA_{20} dendrimer (Fig. S7) in which primary amines on the surface undergo Michael addition with quinone. Other attractive forces may contribute, including π-π stacking, cation-π interaction, H-bonding, metal coordination complex etc [3–5] depending on the nature of substrate surface. Lap shear investigation shows the adhesion strength was dependent on the type of substrate: collagen > PMMA > glass (Fig. 3d), though the wettability of water on glass (contact angle 27°) [31] is much higher than that of collagen and PMMA surfaces (contact angle 63° and 68.4° respectively) [32,33]. The stronger interfacial interaction between collagen (covalent linkage, H-bonding, π-π stacking) with PEI-DBA_{20} adhesive contributes to the higher adhesion strength. The higher adhesion strength on PMMA surface is speculated to be due to the van der Waals force of attraction, H-bonding and partial polymeric diffusion of PEI-DBA_{20} into PMMA surface, whereas there exists only weaker interfacial interaction between glass surface (H-bonding) and PEI-DBA_{20} adhesive. The lap shear experiment results revealed substrate failure for collagen film while mixed mode failure occurred for PMMA substrate (appeared to be predominantly adhesive failure), indicating cohesion energy of the adhesive is stronger. It is speculated that the adhesion strength could be further enhanced by prolonging the curing period, using high molar mass PEI, or mixture of branched and linear PEI.

The cured PEI-DBA_{20} adhesive exhibits viscoelastic character and tolerates an applied shear strain up to 50% with no modulus deviation (LVR in Fig. 3a). Higher strains up 100% displays strain hardening. This implies that the PEI-DBA_{20} coated material would be ductile and will resist to develop crack under applied low shear strains.

In the wake of COVID-19, there has been a heightened need for antimicrobial coated surfaces due to their ability to proactively combat the spread of microorganisms, providing an additional layer of protection and reducing the risk of infection transmission in various settings such as public spaces, healthcare facilities, transportation systems, and other shared environments. Synthetic polycations have been reported to exhibit contact based bactericidal activity against a wide variety of airborne and water borne pathogens when covalently bonded to materials like glass [34–37],

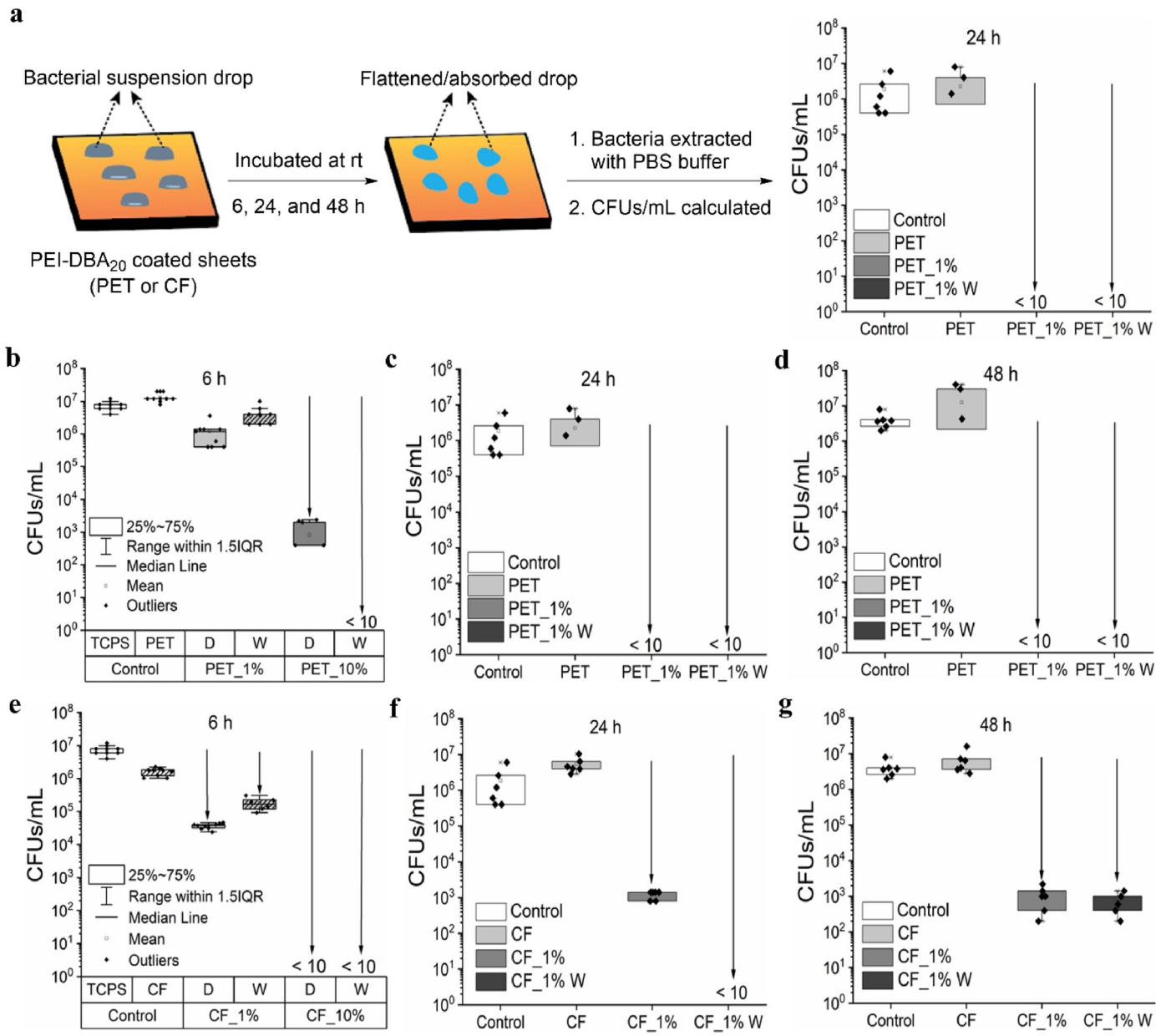

Fig. 5. (a) Antimicrobial study performed with PEI-DBA_{20} coated PET sheets or cloth fibre (CF). Bacterial suspension of *E. coli* was placed on sheets and incubated at room temperature for varied time periods (6, 24, 48 h) followed by counting the number of live bacteria (CFUs/mL) survived. (b) Comparison of the live bacteria represented by CFUs/mL after 6 h incubation upon control (PET) and coated PET sheets (PET_1% & PET_10% dip coated with 1% and 10% PEI-DBA_{20}, D represents dry, W represents washed sheets (with PBS) to remove unbound PEI-DBA_{20}) (n = 9). Comparison of the live bacteria represented by CFUs/mL after: (c) 24 h incubation (d) 48 h incubation (Control represents tissue culture plate, PET represents PET control, PET_1% represents sheets coated with 1% PEI-DBA_{20}, PET_1% W represents washed sheets (with PBS) to remove unbound PEI-DBA_{20}) (n = 6). (e) Comparison of the live bacteria represented by CFUs/mL after 6 h incubation upon neat (Control CF) and coated cloth fiber (CF_1% & CF_10% represent cloth fibre coated with 1% and 10% PEI-DBA_{20}, D represents dry, W represents washed CFs (with PBS) to remove unbound PEI-DBA_{20}) (n = 9). Comparison of the live bacteria represented by CFUs/mL after: (f) 24 h incubation (g) 48 h incubation (Control represents tissue culture plate, CF represents neat cloth fibre, CF_1% represents sheets coated with 1% PEI-DBA_{20}, CF_1% W represents washed sheets (with PBS) to remove unbound PEI-DBA_{20}) (n = 6).

plastics [38], and textiles [35]. These immobilized polycations kill bacteria by damaging their membranes and/or cell walls owing to the positive charge and not by gradually leaching (detaching) from the derivatized surfaces [34]. The materials coated with polycations have been known as self-sterilizing surfaces. However, the methods employed require tedious surface chemistry (chemical derivatization) [35,38], application of harsh reagents [36,37], and elevated temperatures [39]. The most common examples include PEI immobilization on aminosilanized glass surfaces through layer-by-layer glutaraldehyde crosslinking [37], chemical conjugation of PEI to substrates [38], ozone treatment to generate peroxides followed by thermal activation of radical initiation etc [39]. The complex attachment process makes it practically infeasible for routine application or scalable processes. Thus, simple attachment procedures such as painting, dipping or spraying would help overcome abovementioned limitations. The self-crosslinking ability of PEI-DBA_{20} allows for easy coating on the surfaces through methods like dip coating. PEI-DBA_{20} diluted in anhydrous EtOH to coat various substrates, possesses long shelf life at ambient temperature (Fig. S8). The substrate can be dipped in the ethanolic PEI-DBA_{20}

and left at room temperature exposed to atmosphere. Both PEI [40] and anhydrous EtOH are hygroscopic in nature leading to moisture absorption from surrounding atmosphere. The low viscosity of the PEI-DBA_{20} solutions in ethanol would also allow other faster coating methods such as spray coating. Thus, the presented catechol conjugated PEI offers a simple, straightforward method to form self-sterilizing surfaces in scalable and straightforward manner.

The synthesized catechol conjugated PEI is thus proposed for application as antimicrobial coating on the surfaces thereby preventing microbial contamination and bacterial growth. Concentrations as low as 125 μg/mL exhibit inhibition of bacterial growth (MIC) compared to control whereas concentrations ≥2 mg/mL exhibit bactericidal effect. This is in accordance with previous reports wherein pure PEIs (linear and branched) exhibited MIC ranging from 30 to 500 μg/mL against gram negative *E. coli* varying with the molecular weight and molecular weight distribution [27]. The PEIs have also been reported to exhibit antibacterial activity against gram positive *Staphylococcus* with MIC ranging from 8 to 32 μg/mL [27]. However, further investigations are required to assess the antibacterial properties of PEI-DBA_{20} against a spectrum of bacteria. Conjugation of DBA catechol to PEI had no effect on PEI's inherent antimicrobial activity.

Modified static adhesion assay was employed to demonstrate the efficacy of PEI-DBA_{20} antimicrobial coating with PET sheets and clothes. Adhesion-based methods have been employed for evaluating contact-killing designs, particularly for cationic surfaces [41,42]. Static adhesion assays involve exposing test samples to a bacterial suspension, allowing bacteria to settle on the surface while keeping their number below monolayer coverage in contact-killing designs, followed by assessing the number of adhering viable bacteria after a specified time period through careful washing, sonication, and subsequent CFU counting [43–45]. PEI-DBA_{20} coating led to reduction of the contact angle (Fig. 5b, e) suggesting increased hydrophilicity owing to the free $-NH_2$ groups of PEI-DBA_{20}. The increased hydrophilicity of substrates upon coating with cationic polymers like PEI [46], PAMAM [47] is also a common phenomenon. The coated substrates exhibited biocidal activity against *E. coli* a 4–6 log reduction. Washing of the substrate did not remove the biocidal activity (Fig. 5b–g). This suggests that the coatings are resistant to low shear stresses and the antimicrobial activity is due to the cationic surface. Additionally, this would enable the effortless removal of any accumulated dust, debris from dead microorganisms, or similar substances that may adhere to the coating over time, facilitating the reopening of the coated surface through a simple water wash process. However, the polycationic nature of polyethyleneimine (PEI) may raise concerns regarding its potential biotoxicity when considering its application for public use. The surface concentration of PEI-DBA_{20} required for exhibiting antimicrobial activity on PET sheets and cloth fibre was found to be ranging from 0.1 to 0.3 mg cm^{-2} which is about 10,000 times lower compared to lethal dose of PEI in rats (1300–2200 mg/kg, oral), thereby effectively addressing and eliminating concerns regarding biotoxicity. Thus, PEI-DBA_{20} coatings are contact-active surfaces free of biocidal leachates [48–50]. The PEI-DBA_{20} adhesive thus acts as a potential antimicrobial coating agent for long term self-sterilizing surfaces.

Future work will address long term and virucidal applications. Several advantages and limitations of application of PEI-DBA_{20} as antimicrobial coatings are noted. As a platform adhesive it has several advantages: 1) scalable, one step chemical synthesis, 2) simple dip or spray coating, 3) water activated crosslinking, 4) no requirement of harsh chemicals or elevated temperatures, 5) covalent bond formation with substrates, 6) contact-active biocidal mechanism, 7) formation of thin, insoluble polymer complex upon crosslinking. However, several limitations are noted including relatively soft kPa modulus that precludes its use in structure adhesives. Another limitation for coating applications is the color change of the substrate upon crosslinking with higher absorption in the blue-green visible wavelengths. Transparent surfaces turned brown and blue fabrics went almost black (Fig. S2 & S9). The coatings further require assessment in terms of wash cycles and abrasion resistance upon washing for fabric applications. Future work will require further parameter optimization to expand upon its biocidal activity.

5. Conclusions

An auto redox, water-activated adhesive based on PEI dendrimer is demonstrated for antimicrobial coatings. Application is simple since the water-based stimulus is present on most substrates and the PEI dendrimer is naturally hygroscopic. The PEI-DBA_{20} adhesive bonds to a variety of surfaces such as collagen, plastic, and glass. The presence of catechol/Schiff base pair serves as a quinone precursor, a known adhesive crosslinker. Antimicrobial property endowed by multiple amine functional groups of PEI. Through a simple dip process, micron thick coatings were possible on plastic sheets and cloth fibres. Coated substrates displayed contact-active antimicrobial activity against gram negative *E. coli* exhibiting 4–6 log reduction of viable cells. The antimicrobial property could be tuned by varying the concentration of PEI-DBA_{20} coating solution in organic solvent. Adhesive property of PEI-DBA_{20} in coupling with antimicrobial activity makes it a promising material for coating and other biocidal applications.

Credit author statement

Animesh Ghosh: Conceptualization, Data curation, Investigation, Formal analysis, Writing - Original Draft. Juhi Singh: Conceptualization, Data curation, Investigation, Formal analysis, Writing - Original Draft. Sierin Lim: Conceptualization, Review, Funding acquisition. Terry W.J. Steele: Conceptualization, Funding acquisition, Formal analysis, Methodology, Supervision, Writing, Review and Editing - Original Draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to acknowledge the financial support from the Ministry of Education – 'Electrocuring Resins for Advanced Manufacturing' grant RG47/21 (2021-T1-001-077) and 'REDOXIVE: Water activated adhesives and antiviral coatings' grant (T2EP10220-0013) to TWJS. The project is also partially supported by National Research Foundation Biological Design, Tools, and Applications Grant (NRF2013-THE001-046) and A*STAR IAF PP Grant: Wound Care Innovation for the Tropics (H18/01/a0/0P9) to SL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtadv.2023.100398.

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