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A Multifunctional, Low Friction, Antimicrobial Approach for Biomaterial Surface Enhancement

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Abstract

Poly(vinyl chloride) (PVC) biomaterials perform a host of life-saving and life-enhancing roles when employed as medical devices within the body. High frictional forces between the device surface and interfacing tissue, however, can lead to a host of complications including tissue damage, inflammation, pain and infection. We herein describe a versatile surface modification method using multifunctional hydrogel formulations to increase lubricity and prevent common device-related complications. In a clinically-relevant model of the urinary tract, simulating the mechanical and biological environments encountered *in vivo*, coated candidate catheter surfaces demonstrated significantly lower frictional resistance than uncoated PVC, with reductions in coefficient of friction values of more than 300-fold due to hydration of the surface-localised polymer network. Furthermore, this significant lubrication capacity was retained following hydration periods of up to 28 days in artificial urine at pH 6 and pH 9, representing the pH of physiologically normal and infected urine respectively, and during 200 repeated cycles of applied frictional force. Importantly, the modified surfaces also displayed excellent antibacterial activity, which could be

facilely tuned to achieve reductions of 99.8% in adherence of common hospital-acquired pathogens, *Staphylococcus aureus* and *Proteus mirabilis,* relative to their uncoated counterparts through incorporation of chlorhexidine in the coating matrix as a model antiseptic. The remarkable, and pH-independent, tribological performance of these lubricious, antibacterial and highly durable surfaces offers exciting promise for use of this PVC functionalization approach in facilitating smooth and atraumatic insertion and removal of a wide range of medical implants, ultimately maintaining user health and dignity.

Keywords

Functional surfaces; tribology; biomaterials; medical devices; infection

1. Introduction

Poly(vinyl chloride) (PVC) is one of the most widely employed biomaterials in medical implants and devices, including endotracheal tubes, urinary catheters and medical tubing. The diverse healthcare applications result from its relatively low cost, biological and chemical stability, and ready processability [1]. Insertion and removal of these devices can, however, adversely affect tissue integrity as a result of high frictional forces between the interfacing surfaces [2]. While the tribological behavior against human tissues of artificial limbs, fraction fixation devices, and joint, cardiovascular and dental implants has been extensively studied, the tribology of urinary catheter surfaces has, in contrast, received comparatively less attention to date, even though urinary catheters are the most frequently deployed medical device in clinical care [3].

Urinary catheters may be inserted intermittently to drain urine from the bladder when required, or remain *in situ* for up to three months between scheduled device changes when employed for the long-term management of incontinence [4]. Patients who undergo repeated catheterization with poorly lubricated indwelling and/or intermittent devices have, however, been reported to experience urethral bleeding, trauma, pain and inflammation as a result of high frictional forces between the biomaterial surface and interfacing tissues, which can ultimately result in infection and formation of urethral strictures [5-8].

Increased lubricity of the device surface is anticipated to prevent associated pain and tissue trauma by decreasing frictional forces between the catheter surface and the urethral tissue upon device insertion and removal [9]. A common approach to promote smooth catheterization and reduce interfacial friction involves the application of lubricating agents or jelly-like materials, for example glycerin or lidocaine gels [10]. An alternative strategy to enhance usability of these devices and ensure sufficient robustness of the lubricious surface throughout the entire catheterization process involves the application of hydrophilic coatings [5, 11, 12]. These coatings have been widely applied to a range of implantable medical devices, such as endovascular catheters and guidewires, to minimize soft tissue damage upon interfacial contact with the vessel wall or urethra [5, 13]. As a result of their lubricious properties they have also been applied in many industrial applications to reduce frictional forces and associated component wear [13]. Previous studies involving both *in vitro* and *in vivo* models for investigation of frictional properties of catheter surfaces have reported significantly lower interfacial frictional forces with the use of hydrated hydrophilic-coated catheters relative to their uncoated counterparts [11, 12, 14, 15]. This effect is, however, commonly

short-lived due to ready delamination of the coating as a result of high interfacial stresses between the device surface and the urethral tissue [5].

In addition to the reported tissue damage, catheter surfaces also provide an attractive niche for colonization of infecting pathogens, of which the urease-producer *Proteus mirabilis*, which has been identified as one of the most common causes of catheter-associated urinary tract infections, is a notable example [16, 17]. Infection with this pathogen results in elevation of urinary pH to levels up to pH 9.1 as a result of urease-catalysed hydrolysis of urea in the urine to ammonia, leading to precipitation of calcium and magnesium ammonium phosphates [18, 19]. Accumulation of these crystals within the surface-attached bacterial biofilm leads to recurrent blockages of the catheter lumen in a reported 50% of all chronically catheterized patients, necessitating device replacement and, furthermore, increasing the risk of urinary retention and associated problems of pyelonephritis and septicemia [20]. The development of lubricious and durable surfaces which resist bacterial colonization and retain their friction-lowering properties within environments of varying pH is therefore urgently needed. Despite the wide variation in urinary pH, resulting not only from infection but also from diet and drug therapy, the effect of pH on the tribological performance of coated catheter surfaces has, until now, never been examined [21, 22].

Herein, we describe the development of a robust and widely-applicable method to enhance functionality of PVC surfaces and resultant medical devices, such as intermittent urinary catheters. The tribological and microbiological performance of the modified surfaces was evaluated under mechanically- and biologically-relevant conditions to inform the capacity of this approach for reducing frictional forces and

ultimately preventing device-associated tissue trauma, infection and pain. PVC, representing the most widely employed biomaterial, was coated with a hydrogel formulation by a dip coating and UV curing process. Coefficients of friction (COF) of the modified surfaces were measured using a biologically-representative *in vitro* model of the tribological conditions experienced in the urinary tract *in vivo* after varying periods of incubation in deionized water (dH_2O) and artificial urine of pH 6 and pH 9, representing normal and infected urinary pH respectively, against a polydimethylsiloxane (PDMS) probe. The tribological behaviour of the coated surfaces was, in addition, evaluated over 200 repeated frictional cycles to assess coating durability. Resistance to bacterial colonization was studied by challenging modified surfaces with the Gram-negative urinary pathogen *P. mirabilis* and the common Gram-positive nosocomial pathogen *Staphylococcus aureus.*

2. Materials and methods

The coating components: ethylene glycol dimethacrylate (EGDMA), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, 2-hydroxyethyl methacrylate (2-HEMA), chlorhexidine and isopropanol; the artificial urine components: potassium dihydrogen orthophosphate, magnesium chloride hexahydrate, urea, calcium chloride dihydrate and chicken ovalbumin; and the neutralizer components: Tween 80 and azolectin, were obtained from Sigma-Aldrich (Dorset, UK). Kollidon 90F was supplied by BTC Europe (Germany). Poly(vinyl chloride) (PVC) sheets (unplasticised, 0.2 mm thickness) were purchased from Goodfellow Ltd. (Cambridge, UK). Phosphate-buffered saline (PBS), quarter-strength Ringer's solution (QSRS), tryptone soya broth (TSB), agar, glycerol and Mueller-Hinton broth (MHB) were obtained from Oxoid Ltd.

(Hampshire, UK). *Proteus mirabilis* ATCC 35508 and *Staphylococcus aureus* ATCC 6538 (LGC Standards, Middlesex, UK) were maintained on cryopreservative beads (Protect Bacterial Preservation System, Technical Service Consultants Ltd., UK) in 10% glycerol at -80°C and cultured by inoculation into MHB and incubation at 37°C when required for the *in vitro* microbiological assessments.

2.1 Preparation of hydrogel-coated PVC

The coating formulation with a final component composition of 37% w/v was prepared by dissolving the crosslinker EGDMA (3.7 g L^{-1}), the hydrogel monomer 2-HEMA (248 g L^{-1}), the thickening agent Kollidon 90F (111 g L^{-1}) and the photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (7.4 g L^{-1}) gradually, with stirring at ambient temperature, in a solvent mixture of isopropanol:water (2:1), under protection from light. In addition, chlorhexidine-loaded hydrogel (CLH) coatings were prepared for *in vitro* microbiological testing by the incorporation of chlorhexidine (1 g L^{-1}) to the previously described coating formulation. Substrates of PVC, as a model biomedical and catheter material, were etched in ethanol for 30 sec and coated by a dip coating procedure using a withdrawal speed of 200 mm min^{-1}. Samples were then dried at 60°C for 20 min and cured by irradiation with UVA light.

2.2 Preparation of artificial urine

Artificial urine (AU) was prepared using a composition based on that described by Cox *et al.* (1987), with the components listed in Table 1 [23]. Two solutions (A and B) were prepared and mixed immediately before use in order to avoid precipitation of calcium phosphate in the form of brushite, which would lead to a corresponding reduction in the concentration of calcium and phosphate ions.

Table 1: Composition of Artificial Urine

Solution A Components	**Mass (g L^{-1})**	**Solution B Components**	**Mass (g L^{-1})**
Potassium dihydrogen orthophosphate	7.6	Calcium chloride dihydrate	3.6
Magnesium chloride hexahydrate	3.6	Chicken ovalbumin	0.2
Urea	16		

AU was adjusted to pH values of pH 6 and 9 to simulate the pH of normal physiological and infected urine, respectively, by the addition of 1 M HCl or NaOH, and sterilized by membrane filtration (pore size of 0.45 μm).

2.3 Surface characterisation

2.3.1 Fourier transform infrared (FTIR) spectroscopy

Functional groups of uncoated and coated sample surfaces were characterized by attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy using a Perkin-Elmer Spectrum Two spectrophotometer equipped with a diamond ATR accessory at a resolution of 4.0 cm^{-1}. Spectra are an average of 128 scans recorded over the range 4000 – 650 cm^{-1} [1].

2.3.2 Contact angle analysis

Static contact angles of dH_2O droplets (4.0 μL) on the surface of coated and uncoated PVC substrates were measured using an Attension Theta optical tensiometer (Biolin Scientific, Sweden) mounted with a digital video camera and OneAttension software for image analysis. Samples were previously hydrated for 24 h in dH_2O and values are reported as the mean ± standard deviation of five measurements collected 1 to 1.5 sec following droplet placement on ten replicate samples.

2.3.3 Kinetics of coating hydration and dry-out

The kinetics of coating hydration and dry-out at ambient temperature were determined by measuring coating thickness from cross-sectional images acquired using a Keyence VHX-2000E microscope every 10 min for 4 h, with a final measurement at 24 h, during hydration in, or following removal from, respectively, an aqueous solution of Toluidine blue O (TBO). Mean ± standard deviation values were calculated from a minimum of five measurements at each time point.

2.4 Tribological assessment

Pin-on-plate reciprocating sliding friction tests on coated and uncoated PVC substrates were performed with an Anton-Paar Nano Tribometer in conjunction with InstrumX software. In this case, the counter surface pin was a PDMS hemisphere (Sylgard 184, DowCorning, USA) with a diameter Ø of 6 mm and surface roughness $R_a < 20$ nm. Figure 1 shows a schematic of the test arrangement used in this study.

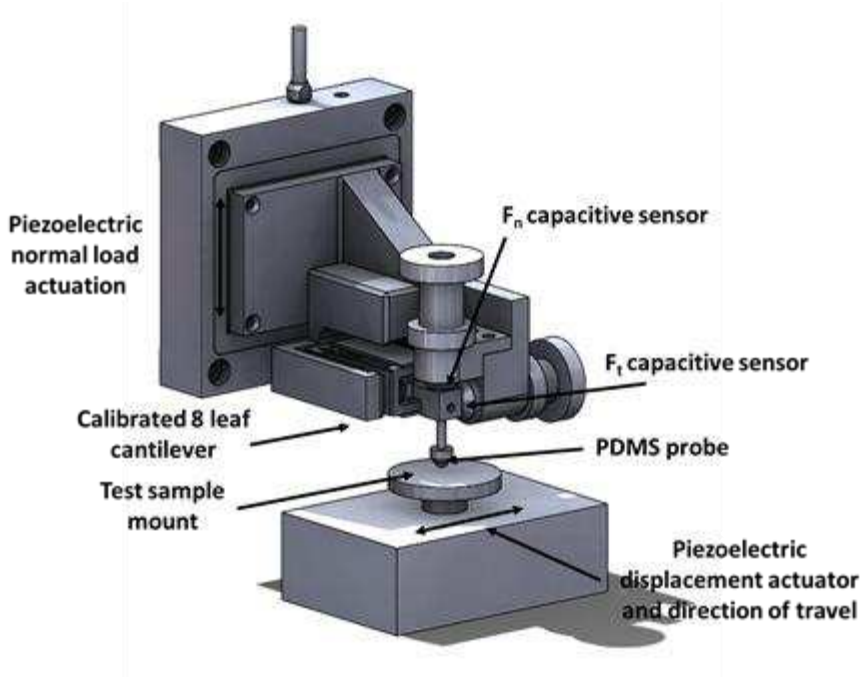

Figure 1. A schematic of the tribometer test arrangement.

The frictional properties of control and coated surfaces were quantified following incubation in dH_2O and AU (pH 6 and pH 9) for 30 sec, 24 h, five days and 28 days. Immediately before tribological assessments, 100 µL of the relevant media (dH_2O or AU) was dropped onto the surface as a lubricant. The PDMS counter probe was slowly lowered until a monotonic increase in force and then zeroed to mitigate any additional buoyancy forces. A normal load of 10 mN was applied to the coated and uncoated PVC surfaces. Sliding was simulated via reciprocation of the PVC surfaces and the frictional forces quantified as a function of cycle position. The tests were conducted at a constant sliding speed of 2 mm/sec at a sliding amplitude of 1 mm for 200 cycles, representing a total sliding distance of 400 mm. This exceeds the distances expected during urinary catheterization, which are reported to be in the region of 40 and 200 mm for females and males respectively[24]. The instantaneous coefficient of friction (COF), µ, was calculated by taking an average of frictional forces measured within the middle 20% of forward and reverse portions of the sliding cycle. The mean steady state COF was

determined from data from the last fifty cycles when the transient behavior had stabilized. Each tribological test was performed with five replicate samples to obtain the mean ± standard deviation values.

2.5 Microbiological assessment

2.5.1 Preparation of challenge inocula

Overnight broth cultures of respective bacteria, *P. mirabilis* and *S. aureus,* were centrifuged at 3000 *g* for 10 min and, after discarding the supernatant, the resulting bacterial pellet was re-suspended in PBS to an optical density equivalent to an inoculum concentration of 1 x 10^8 cfu mL^{-1}, as verified by viable count.

2.5.2 Neutralizer toxicity and efficacy tests

A neutralizer composed of Tween 80 (30 g L^{-1}), azolectin (3 g L^{-1}) and deionized water was prepared for use in microbiological assessments of the CLH coatings to inhibit any residual antibacterial agent and ensure no inhibitory effects on microorganisms recovered from the sample surfaces, following the designated periods of bacterial challenge. Firstly, absence of toxicity of the neutralizer towards the bacterial cells was confirmed by adding 1 mL of the bacterial suspension (1 x 10^8 cfu mL^{-1}) to the neutralizer solution (9 mL), vortexing and counting viable cell density after 10 min incubation. Controls were prepared using PBS in place of neutralizer. Viable counts of the bacterial suspensions were performed by serial dilution, with plating onto MHA and low-swarm agar for *S. aureus* and *P. mirabilis,* respectively. The neutralizer was considered non-toxic if colony counts for the test suspensions were $\leq$1 $\log_{10}$ lower than the counts observed for the control suspensions.

Secondly, efficacy of the neutralizer in quenching the activity of chlorhexidine was tested by adding 1 mL chlorhexidine solution (1% w/v) to the neutralizer solution (8 mL). The solution was vortexed and after 10 min, 1 mL of the bacterial suspension (1 x 10^8 cfu mL^{-1}) was added, vortexed again and incubated for 10 min. Controls were similarly prepared with the use of PBS instead of neutralizer. Viable counts of the test and control bacterial suspensions were performed and the neutralizer was considered effective if $\leq$1 $\log_{10}$ reduction was observed in the neutralized bacterial suspension [25].

2.5.3 Bacterial adherence assessments

Resistance of the hydrogel- and CLH-coated surfaces to bacterial colonization was assessed by challenging coated samples with *P. mirabilis* and *S. aureus* by incubation in infected AU. Suspensions of the respective bacteria with density of 1 x 10^8 cfu mL^{-1} were prepared in PBS as described above and a 1 in 100 dilution was carried out using AU supplemented with 0.5% TSB. Coated samples (1 cm x 1 cm) and uncoated PVC, as controls, were placed into individual wells of a sterile 24-well flat bottom tissue culture plate (Corning Inc., Corning, NY). Aliquots of bacterial suspension (1 mL) with a density of 1 x 10^6 cfu mL^{-1} were added to each well and the plates were shaken in an orbital incubator at 100 rpm at 37°C. After designated 4 h or 24 h incubation periods, samples were removed from the bacterial suspension and rinsed three times in QSRS to remove non-adherent bacteria. Adherent bacteria were then removed by sonicating samples for 10 min in neutralizer solution and vortexing for approximately 30 sec. The sonication technique has previously been demonstrated not to affect bacterial viability or morphology[26]. Viable counts of the resulting neutralizer solution were performed by the Miles and Misra serial dilution technique, with plating onto MHA and low-swarm agar for subsequent enumeration of adherent *S. aureus* and *P.*

mirabilis, respectively, per sample [27]. Five replicates were assessed for each period of incubation and numbers of adherent bacteria on the coated samples have been expressed as percentage values relative to uncoated PVC controls [28].

2.6 Statistical analysis

Statistical differences in COF values and sessile contact angles between coated surfaces and uncoated PVC controls were evaluated by an unpaired t test. Time-dependent measurements of coating thickness during hydration and dry-out were statistically analysed by a two-way analysis of variance. Resistance to bacterial colonization between hydrogel-coated, CLH-coated and uncoated PVC controls was evaluated by a one-way analysis of variance, whereas the effect of media pH and duration of hydration on COF values of the coated surfaces was statistically assessed by a two-way analysis of variance. Post-hoc comparisons between means of individual groups were performed using Tukey's honestly significant difference (HSD) test and in all cases differences were considered significant when $p < 0.05$. All statistical calculations were performed with the use of GraphPad Prism 7 software.

3. Results and discussion

The findings from the experimental work are reported below and their relevance discussed to inform widespread use of this surface modification technique for engineering low friction, infection-resistant medical devices for optimal clinical use within environments of varying pH.

3.1 Characterisation of coated surfaces

3.1.1 FTIR spectroscopy

Overlaid ATR-FTIR spectra of uncoated PVC, hydrogel-coated PVC and CLH-coated PVC are presented in Figure 2.

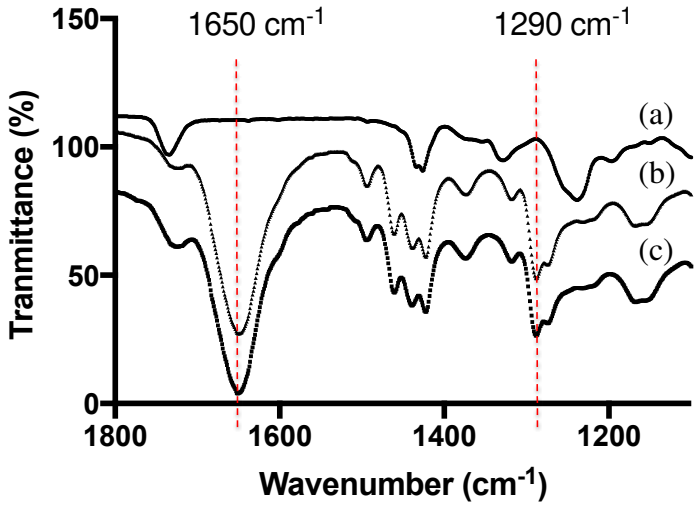

Figure 2. ATR-FTIR spectra of (a) uncoated PVC, (b) CLH-coated PVC and (c) hydrogel-coated PVC from 1800 - 1100 cm^{-1}. Spectra have been offset vertically for clarity.

Spectral differences due to the presence of the coating include additional bands at 3400 – 3300 cm^{-1}, 1650 cm^{-1} and 1290 cm^{-1} attributed to stretching vibrations of O-H, C=O and C-N moieties, respectively, within the HEMA and PVP components at the coated surface [29]. Additional bands within the spectra of the coated PVC at 2930 cm^{-1} and 1450 - 1420 cm^{-1} were ascribed to the alkyl backbones of HEMA and PVP moieties within the coating [30].

3.1.2 Contact angle analysis

To investigate the effect of these differences in chemical composition on wettability of the surface, the angles made by droplets of dH_2O in contact with the solid surface were measured and representative images of these angles are displayed in Figure 3.

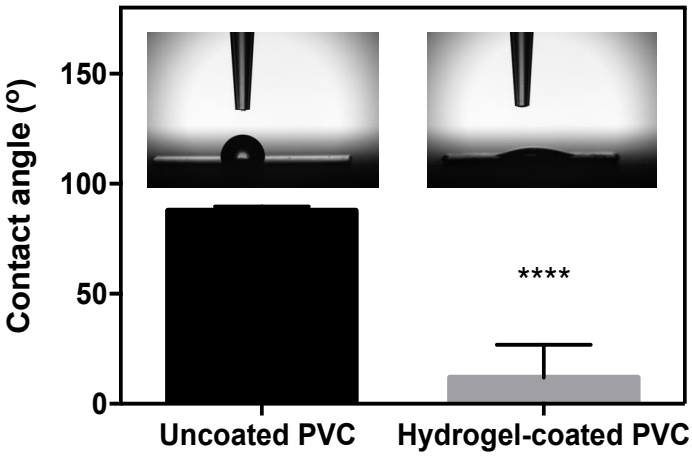

Figure 3. Contact angles of dH_2O on surfaces of uncoated PVC and hydrogel-coated PVC. Values represent the mean ± standard deviation of five measurements collected 1 to 1.5 sec following droplet placement on ten replicate samples. **** denotes significant difference in contact angle ($p < 0.0001$).

The significantly lower contact angles measured herein for the coated surfaces relative to their uncoated PVC counterparts (11.9° ± 14.8° and 87.9° ± 1.8° respectively) reveal the enhanced hydrophilicity of the surface upon application of the hydrogel coating, with dH_2O almost completely wetting the coated surface, as shown in Figure 3. This increased wettability was due to the presence of hydrophilic hydroxyl and carboxyl groups of the HEMA and PVP moieties within the coating [13, 31].

Surface wettability has previously been reported to play an important role in resulting lubricity and friction [13, 32]. In addition, a higher degree of protein adsorption and

bacterial adhesion has been observed on surfaces with moderate hydrophobicity (~90°C) relative to their more hydrophilic counterparts due to the promotion of adherence by hydrophobic interactions between the substrate and bacterial membrane [33, 34].

3.1.3 Kinetics of coating hydration and dry-out

Thickness of the coatings was measured at regular intervals during hydration in, and following removal from, an aqueous solution of TBO using optical microscopy. Representative cross-sectional images of hydrogel-coated PVC stained with TBO, and hydration and dehydration profiles of the hydrogel coating at ambient temperature are shown in Figures 4 (a) and (b) respectively.

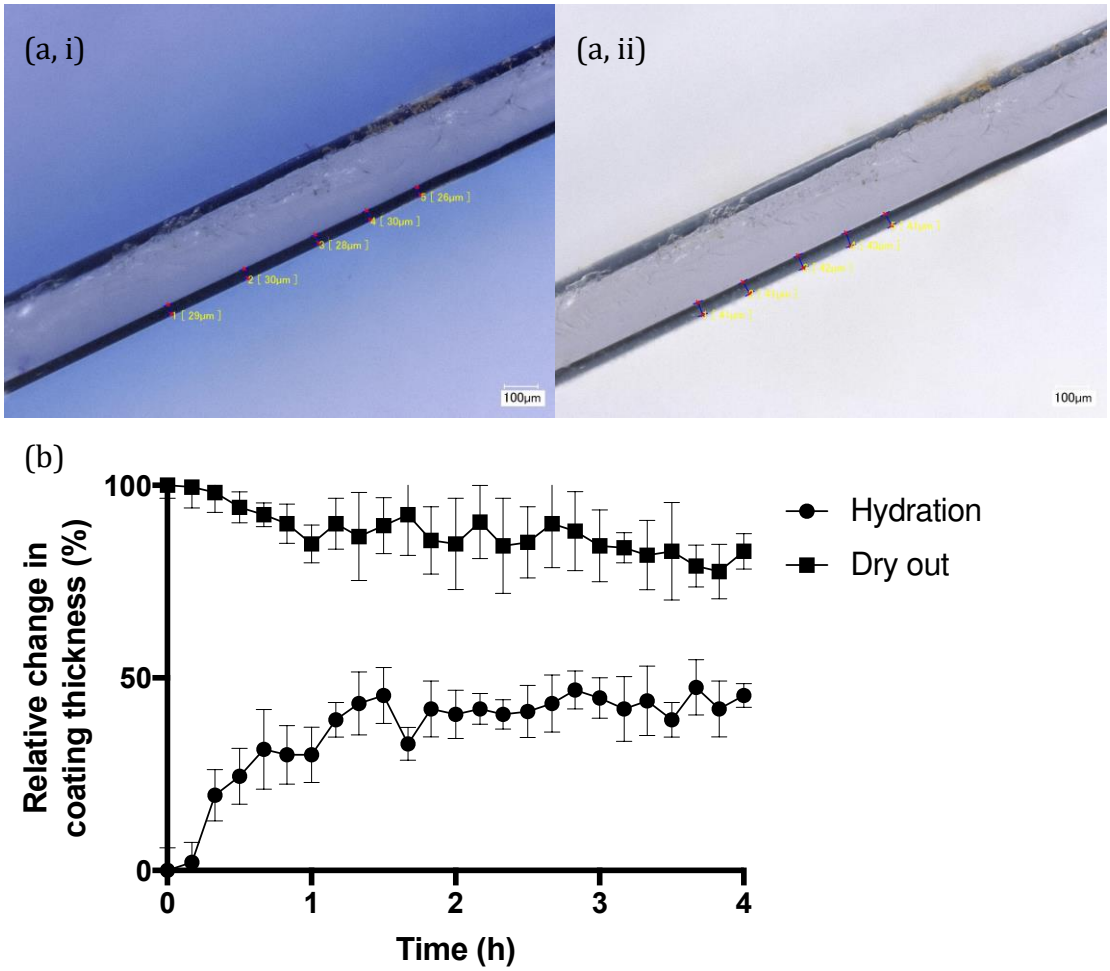

Figure 4. (a) Digital microscopy images of hydrogel-coated PVC (i) 0 h and (ii) 4 h post-hydration at 200x magnification. The coating was stained with TBO dye. (b) Relative change in coating thickness (%) during hydration in, and following removal from, an aqueous solution of TBO. Values displayed are the mean of five replicate measurements and error bars represent standard deviations of the mean values.

The stained hydrogel coating can be seen in Figure 4 (a) as a uniform and distinct layer on the PVC surface. The significant increase in coating thickness upon hydration, as shown in Figure 4 (b), was attributed to the rapid uptake of water and resultant swelling of the hydrogel layer. A rapid and significant increase in coating thickness was observed after a 20 min period of hydration and the values appeared to plateau after 90 min when the thickness of the coating had increased by 45.5%. An increase in surface

lubricity has previously been reported with increased thickness of hydrophilic-coated layers as a result of the greater capacity for imbibement of water [32]. With regards to coating dry-out, no significant differences in coating thickness were observed until 200 min after removal from the wetting media when the coating had decreased in thickness by 15.7%. The slower rates of coating dry-out demonstrate the ability of the hydrogel coating to retain the imbibed water, which is highly beneficial in clinical applications. Rapid loss of water from hydrophilic coatings has previously been found to increase resultant tissue damage as a result of the higher frictional forces between dried-out, 'sticky' coated surfaces and interfacing tissue [35, 36].

3.2 Tribological assessment

Tribological performance of the modified surfaces was assessed by measuring COF values of coated and uncoated PVC surfaces against a PDMS counterprobe under a normal applied load of 10 mN. COF values measured after varying periods of hydration in dH_2O and AU (pH 6 and pH 9) are shown in Figure 5.

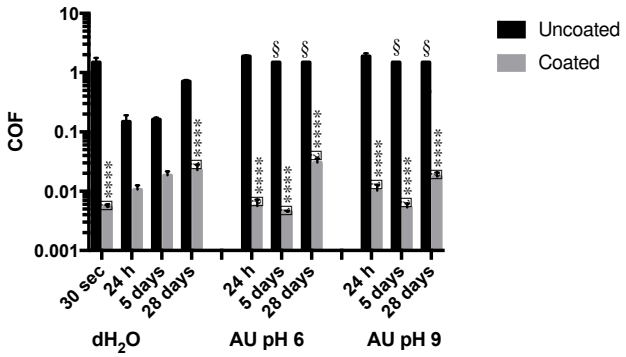

Figure 5. Cycle average COF values of coated and uncoated PVC surfaces following hydration in dH_2O and AU. COF values have been averaged over the last fifty sliding cycles. Columns and error bars represent mean values ± standard deviations ($n \geq 5$). **** denotes significant difference in COF values relative to uncoated control ($p < 0.0001$). [§]COF values were too high to be measured without damaging the counterprobe.

Under all testing conditions, coated PVC surfaces demonstrated significantly lower COF values than their uncoated counterparts, with mean COF values as low as 0.0045 against the PDMS probe following a five-day hydration period in AU pH 6, thereby revealing the lubricious properties of the applied hydrogel coatings. The low frictional forces characteristic of hydrophilic surfaces are commonly associated with the presence of surface-localised lubricating layers of water and the corresponding increase in smoothness and lubricity of the surface [30]. In this case, the significant reductions in COF values relative to uncoated controls were attributed to the presence of the highly solvated hydrogel network resulting from the absorption of water molecules by the hydroxyl and carboxyl groups of the HEMA and PVP moieties [13]. In agreement with previous reports where friction was reported to be dependent on the degree of polymer solvation, this hydrated network was found to modify interfacial contact mechanics leading to lower contact pressures during sliding of the probe over the coated surface, with significant reductions in frictional forces [13, 37, 38].

COF values of the coated surfaces were, in addition, demonstrated to be independent of media pH after up to five days hydration in AU of pH 6 and pH 9. In contrast, rapid swelling of polyacrylic acid (PAA) hydrogels as pH of the wetting media was increased from pH 3 (below the pK_a of PAA) to pH 10 (above the pK_a of PAA) resulted in pH-responsive frictional properties of the artificial cartilage materials, namely high friction and superlubrication in acid conditions and alkaline media respectively [39]. While the effect of pH on tribological performance of hydrophilic catheter coatings has not previously been explored, the pH-independent frictional behavior of the coatings developed herein from the nonionic monomer, HEMA, is of significant importance in consideration of their potential clinical application within the urinary tract, which maintains a normal physiological pH range between pH 4.8 to pH 8, with elevations up to pH 9.1 reported during infection [18, 19].

In order to assess coating stability, tribological tests were performed with samples which had been incubating in dH_2O or AU at 37°C for extended periods of up to 28 days, representing the duration that indwelling urinary catheters may be *in situ* within the bladder between scheduled device changes [35]. Importantly, as shown in Figure 5, the frictional response of coated surfaces was again more than 85-fold lower than for uncoated PVC controls after a four-week incubation period. In the case of immersion in dH_2O (Figure 5), an increased immersion time resulted in an increased COF; although significantly lower than the uncoated surfaces. This may be a result of dissolution of coating components upon prolonged incubation, with an accompanying change in coating integrity, osmotic pressure and resultant lubricity [40]. A similar observation was demonstrated for the coated surfaces immersed in AU (Figure 5), although the trends were not consistent with immersion time or pH.

Furthermore, no significant differences in frictional behavior of the coated surfaces were observed following hydration in AU or dH_2O, despite the osmotic differences between these two solutions. This is in contrast to alternative hydrogel systems of polyvinyl alcohol/polyvinylpyrrolidone (PVA/PVP), where significantly lower COF values were observed after swelling in polyethylene glycol (PEG)-based osmotic solution for 28 days relative to values obtained for their counterparts swollen in a non-osmotic PBS solution. These differences were attributed to deswelling of the PVA/PVP hydrogel in the former ionic environment and the corresponding decreased contact area between the two counterfaces during sliding [40].

Durability of the coated surfaces following hydration in dH_2O and AU for periods from 30 sec to 28 days was assessed using the same tribological parameters outlined above. The COF values of surfaces following incubation in dH_2O and AU have been plotted against cycle number in Figure 6 (a) and (b) respectively.

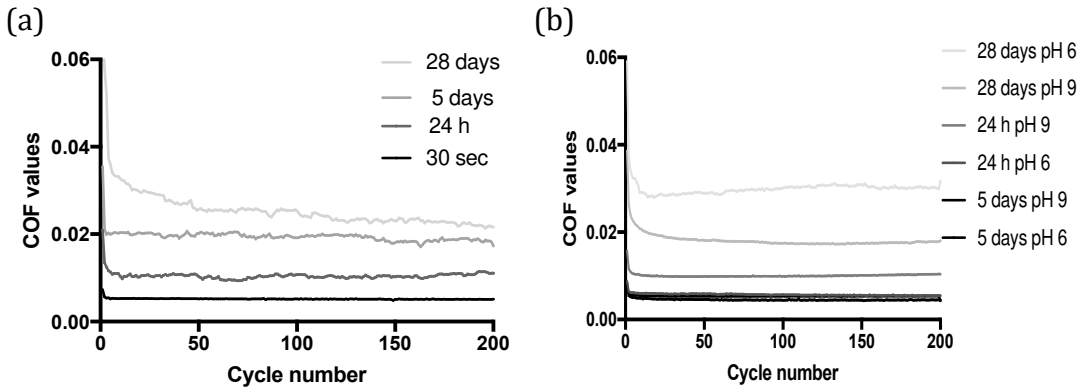

Figure **6.** Mean COF values over 200 repeated sliding cycles on coated sample surfaces following hydration in (a) dH_2O and (b) AU. Error bars have been omitted for clarity however SD values were on average 7-fold lower than their associated mean values.

Coating delamination would be expected to cause an increase in frictional forces, with COF values ultimately approximating those obtained for uncoated surfaces [5, 39, 41]. In contrast, as shown in Figure 6, the COF values remained relatively stable for the duration of all tests, highlighting the impressive wear performance of the coated surfaces [42]. With increasing incubation time, an increase in the COF was seen for surfaces 'aged' in dH_2O (Figure 6 (a)). A similar observation was also seen for surfaces 'aged' in AU, albeit to a higher extent compared to sample surfaces hydrated in dH_2O. In addition to time, the pH of the AU was also seen to affect the COF after extended periods of immersion time. These difference with time and hydration media may be related to degradation of films and exposure to charged species within the different hydration media. These factors could affect the mechanical and swelling properties of the coatings, subsequently affecting the tribological properties. Further work will be conducted to elucidate the links between coating degradation and tribological properties.

3.3 Microbiological assessment

Chlorhexidine, as a model antiseptic, was incorporated within the coating formulation to impart antibacterial properties and reduce the risk of device-associated infections [43]. Neutralizer toxicity testing revealed that the prepared neutralizer was not toxic to *P. mirabilis* or *S. aureus*, with ≤ 1 $\log_{10}$ reductions in the test suspensions relative to the controls as shown in Table 2. Importantly, the neutralizer was also shown to effectively quench the activity of chlorhexidine, with ≤ 1 $\log_{10}$ reductions in bacterial densities observed in the neutralized suspensions of chlorhexidine.

Table 2: Viable Counts from Neutralizer Toxicity and Efficacy Testing

Test solution	***P. mirabilis* (log_{10} cfu mL^{-1})**	***S. aureus* (log_{10} cfu mL^{-1})**
Inoculum density at t = 0 h	7.21 ± 0.065	7.51 ± 0.020
Toxicity test	7.40 ± 0.115	7.36 ± 0.125
Toxicity control	7.26 ± 0.038	7.32 ± 0.046
Efficacy test	7.31 ± 0.137	6.89 ± 0.058
Efficacy control	<1.7	<1.7

Device-associated infections and associated biofilm formation result from the initial adhesion of bacteria to implant surfaces [44, 45]. To establish the capacity of the CLH-coated surfaces to prevent catheter-associated urinary tract infections, we herein evaluated the *in vitro* resistance of hydrogel-coated PVC and CLH-coated PVC to adherence of *P. mirabilis* and *S. aureus* relative to uncoated PVC in AU media. Samples of each material were challenged with inoculi of *P. mirabilis* and *S. aureus* (1 x 10^6 cfu mL^{-1}) over incubation periods of 4 h and 24 h. Bacterial adherence to surfaces of the coated samples relative to uncoated PVC controls is displayed in Figure 7.

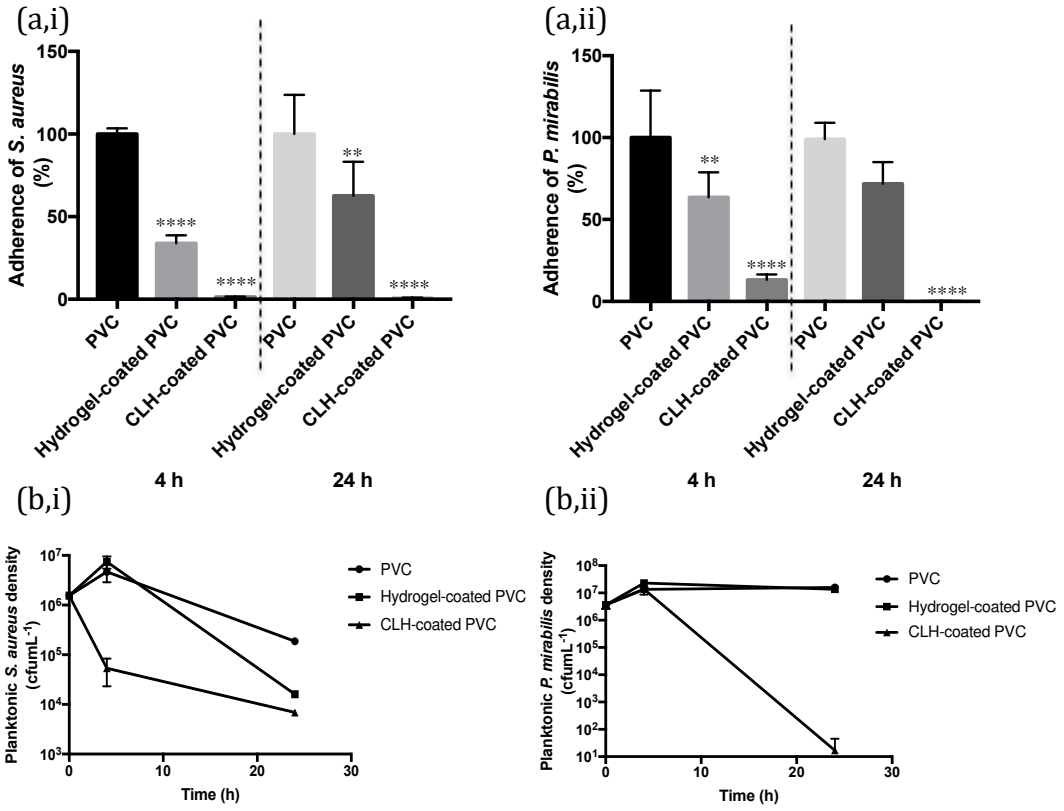

Figure 7. (a) Adherence (%) of (i) *S. aureus* and (ii) *P. mirabilis* to the hydrogel- and CLH-coated PVC samples relative to uncoated PVC controls after 4 h and 24 h incubation at 37°C. Columns and error bars represent mean values ± standard deviations ($n \geq 5$). *Denotes significant reduction in bacterial adherence relative to PVC control (**$p < 0.01$, ****$p < 0.0001$). (b) Viable counts of planktonic bacterial suspensions of (i) *S. aureus* and (ii) *P. mirabilis* after 4 h and 24 h incubation at 37°C in the presence of PVC, hydrogel-coated PVC and CLH-coated PVC. Error bars represent standard deviations of the mean values.

As shown in Figure 7 (a), significant reductions in bacterial colonization of up to 66% were observed on hydrogel-coated PVC relative to their uncoated counterparts. Reduced adherence of a range of common nosocomial pathogens, including *S. aureus, Pseudomonas aeruginosa* and *Escherichia coli,* to hydrophilic-coated surfaces relative to uncoated catheter substrates has previously been reported [30, 46, 47]. This lower degree of bacterial colonization is a result of the hydration layer formed from interactions of the hydrogel chains with water, which effectively acts as a hydrophilic barrier to

bacterial adhesion [34]. Moreover, Figure 7 (a) demonstrates that incorporation of chlorhexidine provided an effective mechanism of increasing resistance of the coated surfaces to bacterial adherence, with significant logarithmic reductions in adherence of *S. aureus* and *P. mirabilis* relative to PVC controls approximating 2.42 and 2.72, respectively, after 24 h incubation. This resistance was attributed to the surface-localised release of chlorhexidine, a cationic bisbiguanide antiseptic, with reported bactericidal activity against both Gram-positive and Gram-negative bacteria [43]. Chlorhexidine functions by disruption of bacterial membranes. Insertion of this agent between phospholipid headgroups, with associated displacement of membrane-localised divalent cations, Mg^{2+} and Ca^{2+}, leads to loss of membrane fluidity and osmoregulation, and at higher concentrations loss of structural integrity and cellular contents [48].

In addition to their observed resistance to bacterial adherence, the CLH-coatings formulated herein demonstrated significant antimicrobial activity against the planktonic bacterial cultures, as shown in Figure 7 (b). After 4 h incubation with CLH-coated PVC, logarithmic reductions of up to 2.0 in the planktonic *S. aureus* suspension were achieved relative to the bacterial population in contact with the uncoated PVC, and after 24 h the logarithmic reductions in planktonic *P. mirabilis* approximated 6.0 in the presence of CLH-coated PVC. Chlorhexidine solutions and dressings are routinely used to cleanse central venous catheter exit sites towards the prevention of catheter-associated bloodstream infections and, while similar solutions have been used for periurethral cleansing before indwelling catheterization, there are to-date no marketed catheters containing this agent localised at the surface [49-51]. The facile manipulation, drug incorporation and drug-releasing capabilities of the lubricious

surfaces engineered herein offer exciting potential for increasing resistance of biomaterial surfaces to colonizing bacteria and ultimately preventing associated infections.

4. Conclusions

The multifunctional biomaterial surfaces engineered herein displayed significant tribological performance, with COF values more than 300-fold lower than uncoated PVC. This behavior was, furthermore, independent of pH and, importantly, maintained after prolonged incubation and repeated applications of frictional force. In addition, microbiological testing confirmed the promising capacity of the CLH-coated surfaces to resist colonization of common clinical pathogens. These findings thereby highlight the promising potential of this robust and widely applicable surface modification approach to reduce device-related complications of tissue trauma, pain and infection.

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Disclosures

The authors declare no competing financial interests.

Data availability

Supporting data are openly available at https://doi.org/10.17034/d5148116-3dcf-4998-b75d-0ef99dd650f2.

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