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Characterisation and evaluation of novel surfactant bacterial anti-adherent coatings for endotracheal tubes designed for the prevention of ventilator-associated pneumonia

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Abstract

It is accepted that ventilator-associated pneumonia is a frequent cause of morbidity and mortality in intensive care patients. This study describes the physicochemical properties of novel surfactant coatings of the endotracheal tube and the resistance to microbial adherence of surfactant coated endotracheal tube polyvinylchloride (PVC). Organic solutions of surfactants containing a range of ratios of cholesterol and lecithin (0:100, 25:75, 50:50, 75:25, dissolved in dichloromethane) were prepared and coated onto endotracheal tube PVC using a multiple dip-coating process. Using modulated temperature differential scanning calorimetry it was confirmed that the binary surfactant systems existed as physical mixtures. The surface properties of both surfactant-coated and uncoated PVC, following treatment with either pooled human saliva or phosphate-buffered saline (PBS), were characterised using dynamic contact angle analysis. Following treatment with saliva, the contact angles of PVC decreased; however, those of the coated biomaterials were unaffected, indicating different rates and extents of macromolecular adsorption from saliva onto the coated and uncoated PVC. The advancing and receding contact angles of the surfactant-coated PVC were unaffected by sonication, thereby providing evidence of the durability of the coatings. The cell surface hydrophobicity and zeta potentials of isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, following treatment with either saliva or PBS, and their adherence to uncoated and surfactant-coated PVC (that had been pre-treated with saliva) were examined. Adherence of *S. aureus* and *Ps. aeruginosa* to surfactant-coated PVC at each successive time period (0.5, 1, 2, 4, 8 h) was significantly lower than to uncoated PVC, the extent of the reduction frequently exceeding 90%. Interestingly, the microbial anti-adherent properties of the coatings were dependent on the lecithin content. Based on the impressive microbial anti-adherence properties and durability of the surfactant coating on PVC following dip coatings, it is proposed that these systems may usefully reduce the incidence of ventilator-associated pneumonia when employed as luminal coatings of the endotracheal tube.

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Introduction

Endotracheal tubes are employed for the artificial ventilation of patients within the intensive care unit. Unfortunately, the use of this medical device in such patients has been implicated in nosocomial (ventilator-associated) pneumonia (Beck-Sague et al 1996; Adair et al 1999). Importantly, nosocomial pneumonia is the second most reported nosocomial infection and accounts for approximately 10–18% of all reported cases (Wenzel 1989; Leedom 1992). Following attachment to the lumen of the endotracheal tube, the invading bacteria secrete an exopolymeric material that encapsulates the parent bacteria within a microbial biofilm (Tunney et al 1998; Gorman & Jones 2002). Existence within the biofilm state offers several advantages to the invading microorganisms (e.g. the resistance of bacteria to antimicrobial agents is dramatically increased in the biofilm state when compared with the planktonic state). In a recent study it was reported that the minimum inhibitory concentration of ceftazidime was at least 1000 times greater toward biofilms of *Pseudomonas aeruginosa* and

Staphylococcus aureus than the comparator planktonic forms of these bacteria (Gorman et al 2001). Furthermore, the microbial biofilm offers greater resistance to the immune response and importantly, microorganisms within the biofilm present on the surface of the lumen may disseminate into the lower respiratory tract, at which point nosocomial pneumonia may develop (Inglis 1990, 1995; Adair et al 1999; Gorman & Jones 2002; Jones et al 2002a). The success of systemic antibiotic chemotherapy for the resolution of ventilator-associated pneumonia is limited due to the increased resistance of microorganisms within the biofilm state and, in addition, the poor distribution of antimicrobial agents within the lumen of the endotracheal tube (Gorman & Jones 2002; Jones et al 2002a). As a result, alternative antimicrobial therapies have been examined for the prevention of ventilator-associated pneumonia, including selective decontamination of the digestive tract (Adair et al 1993; Bergogne-Berezin 1995) and nebulisation of antibiotic solutions to the respiratory tract via the endotracheal tube (Adair et al 2002). However, the clinical efficacy of the former strategy is questionable (Adair et al 1993), whereas nebulisation, while showing early promise (Adair et al 2002), requires further evaluation. Accordingly, to resolve the problems associated with the use of the endotracheal tube, developments are required in the design and function of this class of medical device.

More recently, the use of bioactive coatings has been proposed to reduce the incidence of medical-device-associated infection. Recently, Raad et al (1998) reported that polyurethane catheters that had been coated with minocycline/ethylenediaminetetraacetate were associated with a lower incidence of catheter-related microbial colonisation and bloodstream infections. Similarly, Gabriel et al (1996) observed that the adherence of *Escherichia coli* to a catheter that had been coated with a silver-containing hydrogel was markedly lower than to comparator catheters composed of latex or silicone or to a hydrogel-coated catheter. Maki et al (1997) described a lower incidence of catheter-related infection for silver sulfadiazine/chlorhexidine coated catheters than the non-antimicrobial coated counterpart.

One potential problem associated with the use of antimicrobial coatings on medical devices is the potential emergence of resistant bacteria (Gorman & Jones 2002). Accordingly, an interest has developed in the use of coatings that are inherently resistant to microbial attachment. For this purpose, biodegradable or bio-erodible polymers have been examined, their degradation under physiological conditions facilitating the removal of attached microorganisms. Recently, Jones et al (2002b) described the formulation and characterisation of biodegradable coatings, based on the biodegradable polymer poly(ϵ-caprolactone), that were designed for use as coatings of urinary medical devices. Importantly, the authors illustrated that those coatings that exhibited the greatest rates of degradation also possessed the greatest resistance to encrustation formation. While these coatings have applications for medical devices, their usage is restricted to devices in which there is immersion within a biological fluid, as this medium facilitates the degradation/erosion process. Furthermore, these coatings should be reserved for medical devices in which there is fluid flow adjacent to the device to facilitate removal of the degradation products from the coating. The physiological conditions within the respiratory tract would therefore render the use of biodegradable coatings on endotracheal tubes unsuitable. However, the development of coatings that offer an inherent resistance to microbial colonisation would represent a major development in endotracheal tube design. Therefore, in this study, the physicochemical and microbial anti-adherent properties of surfactant blends were examined as candidate coatings for endotracheal tubes. Knowledge of these properties will allow further conclusions to be generated concerning the potential suitability of these systems for the reduction or prevention of ventilator-associated pneumonia.

Materials and Methods

Materials

Polyvinylchloride emulsion (medical grade) was a gift from Rusch Manufacturing Limited (Lurgan, Northern Ireland). Mueller-Hinton agar and Mueller Hinton broth were purchased from Oxoid (Basingstoke, UK). Lecithin (L-α-phosphatidylcholine) and cholesterol (5-cholesterol-3β-ol) were purchased from Sigma Chemicals Ltd (Poole, UK). Xylene and dichloromethane were purchased from Aldrich Chemical Company (Dorset, UK). All other chemicals were purchased from BDH Chemicals (Poole, UK).

Bacteria isolates and growth

Two bacterial isolates were employed in this study, namely, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These were isolated from microbial biofilm on the surface of clinically retrieved polyvinylchloride endotracheal tubes and maintained on beads or in agar in 10% glycerol at -70°C, as previously described (Jones et al 1997a, 2002a). Stationary-phase bacterial cultures were prepared by transferring samples of each isolate into pre-warmed Mueller Hinton Broth and subsequent incubation at 37°C for 16 h in a carbon-dioxide-enriched atmosphere (5% CO_2/95% air) (Jones et al 1997a, 2001). The bacterial cells were then washed three times with, and finally suspended in, sterile phosphate-buffered saline (PBS, pH 7.3) to the required cell count. When required, bacterial cells were suspended in pooled human saliva (diluted 1 : 1 in sterile PBS) and shaken at 37°C for 30 min before further analysis, as previously reported (Jones et al 1997a, 2001).

Preparation of polyvinylchloride samples and surfactant-coated polyvinylchloride samples

Sheets of polyvinylchloride (thickness 2.0 ± 0.1 mm) were prepared by casting polyvinylchloride emulsion between two glass plates that were separated by metal spacers, and heating at 160°C for 10 min. Following manufacture, any unreacted monomer was removed by rinsing the sheets in distilled water for at least 14 days. Samples of polyvinylchloride were then coated with various surfactant systems

using a dip-coating process. In this, a series of organic solutions were prepared by dissolving a defined mass of surfactants (5% w/w) in dichloromethane with the aid of stirring. Solutions were prepared containing a range of ratios of cholesterol and lecithin, namely 0:100, 25:75, 50:50 and 75:25. Dip coating was performed by immersion of sections of polyvinylchloride into the various surfactant solutions for 5 s. The organic solvent was then allowed to evaporate at room temperature in a fume cupboard and the process repeated four further times. When required, the prepared uncoated and coated polyvinylchloride samples were treated with either PBS or pooled human saliva (diluted 1:1 in sterile PBS) and shaken at 37°C for 30 min before further analysis, as previously reported (Jones et al 1997a, 2001).

Determination of bacterial cell surface hydrophobicity using the Bacterial Adherence to Hydrocarbons (BATH) test

Characterisation of the surface hydrophobicity of the bacterial isolates employed in this study was performed using the Bacterial Adherence to Hydrocarbons (BATH) test, as previously reported (Jones et al 1996, 2001). In brief, stationary-phase bacteria that had been grown in a carbon dioxide (5% v/v)-enriched atmosphere, and treated with either pooled saliva or PBS, were suspended in phosphate buffer (pH 7.4) to a defined optical density (0.7 at 540 nm). A defined volume of the bacterial suspension (4.8 mL) was then added to the model hydrocarbon phase (xylene, 1.0 mL). This disperse system was then vortex mixed for 5 min, allowed to equilibrate for 20 min and the optical density of the aqueous bacterial phase determined (540 nm). The cell surface hydrophobicity is expressed as the percentage of the original absorbance of the bacterial suspension present in the organic phase. All experiments were replicated at least five times.

Characterisation of bacterial zeta potential

The cell surface charge of the bacterial isolates that had been grown to the stationary phase in a carbon-dioxide-enriched atmosphere and treated with either pooled saliva or PBS were determined using a Malvern Zetasizer IV (Malvern Instruments, Malvern, UK) (Jones et al 1997a, 2001). Samples of bacterial suspensions (10^9 colony forming units (cfu) mL^{-1} in PBS) were injected into a ZET 5104 capillary cell at 25°C and measurement of zeta potential determined under defined conditions (field strength 10–20 V cm^{-1}, electrode spacing 50 mm, dielectric constant 78.54). All experiments were replicated at least six times.

Dynamic contact angle analysis of polyurethane and surfactant-coated polyurethane

The advancing and receding contact angles of sections of polyvinylchloride and surfactant-coated polyvinylchloride were determined using a Dynamic Contact Angle Analyser (DCA 312, Cahn Instruments) at 25°C. In this, samples were immersed into reagent-grade-1 water at a defined rate (150 $\mu m\ s^{-1}$) as previously described (Jones et al 1997a, b, 2001). Furthermore, the effect of sonication on the advancing and receding contact angles of surfactant-coated sections of polyvinylchloride was examined. In this, sections of coated polyvinylchloride were immersed in an ultrasonic water bath for 5 min and the advancing and receding contact angles determined as above. All experiments were replicated at least five times.

Thermal characterisation of surfactant coatings

Examination of the thermal properties of the various surfactant coatings was performed using modulated temperature differential scanning calorimetry. In this, samples were prepared by placing the organic solutions of lecithin and lecithin and cholesterol (75:25, 50:50 and 25:75 in dichloromethane) into an aluminium Perkin Elmer DSC pan and allowing the solvent to evaporate overnight. The pans were then hermetically sealed with a pinhole lid. Thermal analysis was performed using a TA Instruments MTDSC 2920 (Leatherhead, Surrey) with a refrigerated cooling system, following calibration with indium. A heating rate of 5°C min^{-1} was applied over a range of −10 to 120°C. In all cases at least triplicate measurements of the relationship between heat flow and temperature were determined for each surfactant system.

Bacterial adherence assay

Discs of polyvinylchloride that had been pre-treated with pooled human saliva were anchored to the bottom of sterile McCartney bottles under aseptic conditions. Into these were added samples of bacterial suspensions (stationary phase, 15 mL, ca. 1×10^7 cfu mL^{-1}) and the bottles incubated at 37°C with shaking in a carbon-dioxide-enriched (5% CO_2) atmosphere. At designated time intervals (0.5 1, 2, 4, 8 h), the sample bottles were removed, the bacteria decanted off and the discs removed using sterile forceps. Non-adherent bacteria were removed from the surface of the discs by vortexing (at low speed) for 30 s in two 5-mL volumes of PBS. Bacterial removal was ensured by plating out selected final rinsings onto Mueller Hinton agar and enumerating viable bacteria using a serial dilution technique (Jones et al 1997a). Absence of viable organisms in the final wash was accepted as indicating successful bacterial removal. At each time interval each disc was placed in PBS (10 mL) and adherent bacteria removed by sonicating at low power for 5 min and vortexing for 30 s (Jones et al 1997a). After removal of the discs, the number of viable bacteria was determined by serial dilution. Sonication was shown not to affect either microbial viability or morphology. Results are expressed as the percentage of the initial inoculum that was adherent to the biomaterial at each time interval (Jones et al 2001).

Statistical analysis of data

The effects of coating composition (uncoated, 0:100, 25:75, 50:50, 75:25 cholesterol–lecithin) on the adherence of *S. aureus* and *Ps. aeruginosa* (expressed as the% of adherent

organisms from the initial inoculum) and the temperature of endothermic events and the associated heat flow were statistically examined using a one-way analysis of variance. Conversely, the effects of coating composition and presence or absence of sonication or, alternatively, the effect of coating composition and treatment with either PBS or pooled human saliva on the advancing and receding contact angles of the various biomaterials were statistically evaluated using a two-way analysis of variance. Finally, the effect of pre-treatment with either PBS or saliva on the surface hydrophobicity and zeta potential of *S. aureus* and *Ps. aeruginosa* were statistically examined using a one-way analysis of variance. Post-hoc comparisons of the means of individual groups were performed using Dunnett's test. In all cases $P < 0.05$ denoted significance.

Results

Pre-treatment with pooled human saliva significantly decreased both the cell surface hydrophobicity and the zeta potential of *S. aureus* or *Ps. aeruginosa* when compared with the PBS-treated comparators (Table 1). Due to the greater initial cell surface hydrophobicity of *S. aureus*, the effects of saliva treatment on this cell surface property was significantly greater than for the more hydrophilic *Ps. aeruginosa*.

The effects of coating composition and sonication on the advancing and receding contact angles of polyvinylchloride are depicted in Table 2. The advancing and receding contact angles of polyvinylchloride were $89.88 \pm 1.45°$ and $65.15 \pm 0.67°$, respectively – values that were statistically unaffected by sonication. Interestingly, polyvinylchloride that had been coated with either lecithin or various combinations of lecithin and cholesterol possessed significantly lower advancing and receding contact angles than native polyvinylchloride. Typically, the range of advancing contact angles exhibited by these systems was $66.85 \pm 4.16°$ to $68.39 \pm 1.99°$, whereas the range of receding contact angles for the same coatings was $58.81 \pm 4.17°$ to $61.60 \pm 2.24°$, the magnitudes of which were unaltered following sonication for 5 min. The ratios of cholesterol and lecithin used as coatings in this study (0:100, 25:75, 50:50, 75:25) did not significantly influence the resultant surface energies (i.e., the advancing and receding contact angles of these coatings were statistically similar). The effect of pre-treatment with saliva on the dynamic contact angles of the various coated and uncoated biomaterials is shown in Table 3. Treatment with saliva significantly lowered the advancing and receding contact angles of uncoated polyvinylchloride, although the contact angles of the coated polyvinylchloride samples were statistically unaffected by pre-treatment with saliva.

Figure 1 presents the thermal properties of the various coating systems employed in this study, as determined using modulated differential scanning calorimetry. The coating composed of lecithin exhibited an endotherm at $23.64 \pm 0.21°$. This endotherm was, however, also observed in coatings containing lecithin and cholesterol; as the

Table 1 The effect of pre-treatment with either saliva or PBS on the surface hydrophobicity and zeta potential of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Treatment	Cell surface hydrophobicity[a]		Zeta potential (mV)	
	S. aureus	*Ps. aeruginosa*	*S. aureus*	*Ps. aeruginosa*
PBS	23.09 ± 0.28	6.12 ± 0.04	-35.35 ± 0.54	-35.99 ± 0.23
Saliva	0.01 ± 0.16	4.73 ± 0.01	-31.33 ± 0.23	-32.98 ± 0.14

[a]Results are expressed as the % of the original inoculum (absorbance) present in the organic phase following equilibration, as described in Materials and Methods.

Table 2 The effects of coating composition and sonication on the mean ($\pm$s.d.) advancing and receding contact angles of polyvinylchloride (PVC).

Coating composition	Advancing contact angle (°)		Receding contact angle (°)	
	No sonication	Sonication	No sonication	Sonication
Uncoated PVC	89.88 ± 1.45	88.32 ± 2.43	65.15 ± 0.67	59.34 ± 1.03
0:100 Cholesterol–Lecithin[a]	68.39 ± 1.99	63.87 ± 3.99	61.60 ± 2.24	60.67 ± 2.34
25:75 Cholesterol–Lecithin[a]	68.14 ± 2.89	67.01 ± 1.98	63.84 ± 2.46	61.22 ± 1.84
50:50 Cholesterol–Lecithin[a]	69.51 ± 2.27	68.22 ± 1.95	61.09 ± 2.84	62.32 ± 1.92
75:25 Cholesterol–Lecithin[a]	66.85 ± 4.16	68.02 ± 2.91	58.81 ± 4.17	60.46 ± 3.21

[a]Ratio of cholesterol to lecithin.

Table 3 The effect of saliva treatment on the advancing and receding contact angles of uncoated and surfactant coated polyvinylchloride (PVC).

Coating composition	Advancing contact angle (°)		Receding contact angle (°)	
	PBS treatment	Saliva treatment	PBS treatment	Saliva treatment
Uncoated PVC	89.88±1.45	70.00±2.34	65.15±0.67	62.38±2.71
0:100 Cholesterol–Lecithin[a]	68.39±1.99	70.45±0.88	61.60±2.24	62.94±1.70
25:75 Cholesterol–Lecithin[a]	68.14±2.89	73.19±2.99	63.84±2.46	68.14±3.89
50:50 Cholesterol–Lecithin[a]	69.51±2.27	72.03±2.50	61.09±2.84	65.04±6.11
75:25 Cholesterol–Lecithin[a]	66.85±4.16	69.28±2.27	62.81±4.17	63.62±5.59

[a]Ratio of cholesterol to lecithin.

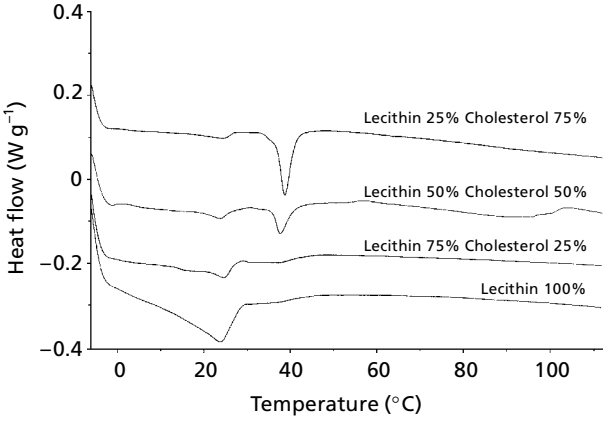

Figure 1 Thermal properties of coatings composed of either lecithin or mixtures of lecithin and cholesterol.

lecithin concentration was decreased, the associated heat flow sequentially significantly decreased. All coatings containing cholesterol possessed an endotherm at 38.06±0.36°, the associated heat flow of which increased as the ratio of cholesterol to lecithin increased. Due to this concentration effect and the absence of this endotherm in the lecithin plot, this thermal event may be assigned to cholesterol.

The effects of coating composition on the adherence of saliva-pre-treated *S. aureus* and *Ps. aeruginosa* to polyvinylchloride and surfactant-coated polyvinylchloride (that had been pre-treated with saliva) at different exposure periods (0.5, 1, 2, 4 and 8 h) are shown in Tables 4 and 5, respectively. In general, increasing the time of exposure of bacteria to polyvinylchloride increased the subsequent adherence, although this time dependency was not observed for each surfactant-coated biomaterial. Importantly, the adherence of *S. aureus* to the various surfactant-coated polyvinylchloride discs was significantly lower than to uncoated polyvinylchloride at each exposure period. Interestingly, the coatings composed of cholesterol–lecithin ratios of 0:100, 25:75 and 50:50 displayed statistically similar reductions in bacterial adherence. Moreover, the extent of this reduction was frequently $\geqslant 90\%$. The adherence of *S. aureus* to polyvinylchloride that had been coated with the 75:25 cholesterol–lecithin ratio, while lower than to native polyvinylchloride, was significantly greater than to the other surfactant coatings that were examined. In a similar fashion, the adherence of *Ps. aeruginosa* to polyvinylchloride that had been coated with cholesterol–lecithin ratios of 0:100, 25:75 and 50:50 was significantly lower than to uncoated polyvinylchloride at each exposure

Table 4 The effect of coating composition on the adherence of *Staphylococcus aureus* to polyvinylchloride (PVC).

Coating composition	Adherence (mean±s.d.) of *S. aureus*[a] to polyvinylchloride or surfactant-coated polyvinylchloride following exposure times of:				
	0.5 h	1 h	2 h	4 h	8 h
Uncoated PVC	0.82±0.11	1.02±0.22	1.12±0.13	1.51±0.22	1.61±0.09
0:100 Cholesterol–Lecithin[b]	0.05±0.01	0.06±0.01	0.07±0.02	0.14±0.04	0.15±0.03
25:75 Cholesterol–Lecithin[b]	0.07±0.01	0.06±0.01	0.1±0.01	0.15±0.02	0.18±0.02
50:50 Cholesterol–Lecithin[b]	0.08±0.02	0.08±0.01	0.1±0.01	0.09±0.01	0.15±0.03
75:25 Cholesterol–Lecithin[b]	0.43±0.09	0.52±0.05	0.56±0.04	0.64±0.08	0.55±0.03

[a]Adherence is expressed as the percentage of the initial inoculum adherent to the biomaterial, as described in Materials and Methods. [b]Ratio of cholesterol to lecithin.

Table 5 The effect of coating composition on the adherence of *Pseudomonas aeruginosa* to polyvinylchloride (PVC).

Coating composition	Adherence (mean±s.d.) of *Ps. aeruginosa*[a] to polyvinylchloride or surfactant-coated polyvinylchloride following exposure times of:				
	0.5 h	1 h	2 h	4 h	8 h
Uncoated PVC	0.21±0.01	0.25±0.02	0.28±0.03	0.41±0.03	0.61±0.05
0:100 Cholesterol–Lecithin[b]	0.02±0.00	0.03±0.01	0.05±0.01	0.04±0.00	0.08±0.01
25:75 Cholesterol–Lecithin[b]	0.03±0.01	0.05±0.01	0.07±0.02	0.07±0.01	0.10±0.02
50:50 Cholesterol–Lecithin[b]	0.11±0.01	0.12±0.02	0.15±0.02	0.30±0.02	0.45±0.05
75:25 Cholesterol–Lecithin[b]	0.13±0.02	0.17±0.02	0.26±0.03	0.36±0.04	0.56±0.03

[a]Adherence expressed as the percentage of the initial inoculum adherent to the biomaterial, as described in the Materials and Methods. [b]Ratio of cholesterol to lecithin.

time. The reductions in adherence to coatings composed of either lecithin or cholesterol–lecithin (25:75) were statistically similar. As the concentration of cholesterol was further increased in the coatings, their resistance to adherence of *Ps. aeruginosa* decreased, the coating composed of a 75:25 ratio of cholesterol–lecithin exhibiting a statistically similar resistance to adherence as to uncoated polyvinylchloride.

Discussion

Several therapeutic strategies have been examined for the prevention of ventilator-associated pneumonia, including selective decontamination of the digestive tract and nebulisation of antimicrobial agents directly to the lumen of the endotracheal tube. However, despite the varied successes achieved to date, it may be argued that to achieve the required clinical performance, advances in the design of the biomaterials for use in the endotracheal tube are required (Jones & Gorman 2002; Jones et al 2002a). Importantly, the endotracheal tube must possess acceptable mechanical properties, thus facilitating insertion and maintaining respiratory patency and furthermore, such devices should offer resistance to the adherence of microorganisms, and hence microbial biofilm formation. The latter requirement may be achieved by the incorporation of antimicrobial agents within the matrix of the endotracheal tube (Jones et al 2002a) and indeed, several authors have reported the clinical success of this approach for the prevention of catheter-related sepsis (Whalen et al 1997; Multanen et al 2000). However, as release of the antimicrobial agent is facilitated by the presence of a surrounding aqueous medium, the success of these systems for the prevention of ventilator-associated pneumonia, in which there is a limited volume of fluid in contact with the lumen of the endotracheal tube, is unclear. Furthermore, the inclusion of antimicrobial agents may compromise the mechanical properties of the endotracheal tube. Recently, it was shown that incorporation of the non-antibiotic antimicrobial agent hexetidine within endotracheal tube polyvinylchloride rendered the biomaterial more resistant to microbial adherence than native polyvinylchloride; the presence of this therapeutic agent was, however, shown to deleteriously affect the mechanical properties of endotracheal tube polyvinylchloride (Jones et al 2002a). This study offered a reminder of the balance that must be achieved between the physicochemical and antimicrobial properties of such biomaterials. Due to the possible effects of therapeutic agents on the mechanical and processing properties of biomaterials, an interest has developed in the use of microbial anti-adherent coatings for the reduction or prevention of medical-device-related infection. While some studies have described the use of coatings with incorporated antimicrobial agents (e.g. Raad et al 1998; Darouiche et al 1999; Johnson et al 1999), other studies have examined the utility of coatings that possess either an inherent microbial anti-adherent activity or an ability to dislodge adherent microorganisms. For example, Bridgett et al (1993) described reduction in the adherence of *Staphylococcus epidermidis* to hydrogel (Hydromer)-coated silicone cerebrospinal fluid shunts in comparison with the non-coated comparator shunts. Similarly, Bambauer et al (1997) examined the in-vivo adherence of bacteria to various polymer surfaces (silicone and polyurethane) that had been treated by ion beam deposition processes. Interestingly, the authors described bacterial colonisation on 2.4% of the surface-treated catheter pieces compared with 7.1% in the non-treated polymer controls. Despite the apparent successes of these approaches, there are problems associated with the use of such coatings, including ease of application and the cost of deposition. Therefore, in this study we have employed a standard dip-coating system that is rapid and cost effective. The choice of surfactants as components of the coating systems employed in this study was based on two major factors. Firstly, the surfactants (lecithin and cholesterol) are pharmaceutically acceptable surfactants that dissolve readily in organic solvents and, secondly, surfactants have been reported to possess microbial anti-adherent properties (Olsson et al 1991; Fowler & Jones 1992; Jones et al 1997c), thus affecting the prequel to microbial biofilm formation.

The initial stage in the colonisation of medical devices (e.g. endotracheal tubes) involves adherence of the invading

microorganism (Jones et al 1997a ; Gorman & Jones 2002). Common to many adherence processes, the surface properties of the bacterium (e.g. cell surface hydrophobicity, zeta potential) and the medical device biomaterial (e.g. surface energy) directly affect their subsequent adherence interaction (Gorman & Jones 2002). Therefore, in this investigation the surface properties of both the microorganisms and biomaterials and, in particular, the effects of environmental conditions on these factors were investigated. The environmental parameters that were chosen in this study, namely growth atmosphere and treatment with saliva, are those that are relevant to the oropharynx (Jones et al 1997a, 2001). The importance of growth atmosphere (i.e. growth within a carbon-dioxide-enriched atmosphere) on microbial cell surface properties and the consequences for microbial attachment to biomaterials have been highlighted in previous studies (Wilcox et al 1991 ; Jones et al 1997a, 2001). Accordingly, a carbon-dioxide-enriched atmosphere was selected as the growth atmosphere in this study. In this study, pre-treatment with saliva decreased the cell surface hydrophobicity and zeta potential of the two microbial isolates. Saliva is a complex biological medium that contains an array of macromolecules, including enzymes, immunoglobulins and glycoproteins, each possessing distinctive physicochemical properties, and which have been reported to adsorb onto both inert and biological substrates (Jones et al 1997a, 2001). Therefore, these alterations in microbial cell surface properties due to the adsorption of salivary macromolecules would be expected to affect microbial adherence to biomaterials.

The coatings examined in this study were chosen for a number of reasons, namely their biocompatibility and their potential microbial anti-adherent properties. In addition, this study has illustrated that the application of these coatings to endotracheal tube polyvinylchloride was straightforward, thereby highlighting another advantage of their potential utility. Characterisation of the states of the cholesterol and lecithin within the various coatings was performed using modulated differential scanning calorimetry. Within the temperature range studied, two primary endotherms were observed at ca. 24°C and the other at ca. 38°C. Due to the effects of component concentration on the heat flows associated with the transitions and the absence of the endotherm at ca. 38°C in the coating composed solely of lecithin, these endotherms may be attributed to lecithin and cholesterol, respectively. Interestingly, the temperature at which the respective endotherms occurred was not influenced by the presence of the other component. Therefore, it may be concluded that in the coatings that contained lecithin and cholesterol, there was minimal interaction between the two components and hence the coatings behaved as physical mixtures. Typically in binary or higher systems in which there are interactions between the various components, the various thermal phenomena (e.g. melting, glass transitions) are markedly modified (Jones 1999).

One surface property of biomaterials that has been reported to influence the subsequent adherence of microorganisms (and hence microbial biofilm formation) is the surface energy (i.e. the hydrophobic/hydrophilic nature) of the material. For example, reduction in the surface hydrophobicity of biomaterials was reported by Weerkamp et al (1988) to modify bacterial adherence, whereas Ferreiros et al (1989) concluded that the adherence of bacteria to a biomaterial is optimal whenever the surface energies of the two surfaces are similar. More recently, Kiremitci-Gumusderelioglu & Pesman (1996) observed that the adherence of a relatively hydrophobic strain of *Escherichia coli* to the hydrophilic surfaces of a series of methacrylate polymers and polyurethane was lower than to the more hydrophobic surface of polypropylene. Furthermore, the reverse was true when a hydrophilic strain of *E. coli* was employed. Therefore, in this study, the advancing and receding contact angles of the various coated and uncoated biomaterials were examined to provide information concerning their hydrophobic/hydrophilic nature. In addition, following insertion, the endotracheal tube will be bathed with saliva, and therefore common to other medical devices, a conditioning film (derived from saliva) will be deposited onto the surface of the device (Jones et al 1997a ; McGovern et al 1997 ; Jones et al 2001). Accordingly, the effects of the deposited salivary conditioning film on the advancing and receding contact angles of the various materials were examined. In accordance with previous studies, uncoated polyvinylchloride exhibited moderately high advancing and receding contact angles (Jones et al 1997a, 2001), the values of which decreased following deposition of the various surfactant coatings. This observation may be accredited to the greater hydrophilic nature of these compounds. Of the two components of the various coatings, lecithin possesses the greater hydrophilic character, as reflected by the higher associated hydrophile–lipophile balance. Therefore, it would be predicted that the advancing and receding contact angles of the various coated biomaterials would decrease as the concentration of lecithin was increased in the coating formulations. However, this study has shown that the concentration of lecithin within the coatings did not affect the subsequent surface energy of the biomaterials and accordingly it may be concluded that the differences in HLB values between the two surfactants did not influence the subsequent behaviour of the molecules at the water–biomaterial interface. Following treatment with saliva, both the advancing and receding contact angles of polyvinylchloride decreased. The ability of saliva to reduce the contact angles of hydrophobic biomaterials (e.g. polyvinylchloride, silicone) has been previously reported (Jones et al 1997a, 2001 ; McGovern et al 1997) and may be accredited to the adsorption of macromolecules from saliva onto the surface of the biomaterial, thereby rendering the surface more hydrophilic. Interestingly, following treatment with saliva, the advancing and receding contact angles of the various coated biomaterials were unchanged. Due to the evidence from previous publications, it is unlikely that these observations may be explained by failure of salivary macromolecules to adsorb onto the surface of the surfactant-coated biomaterials. Therefore, it may be postulated that adsorption of macromolecules occurred, although the mass of materials adsorbed onto coated polyvinylchloride following the treatment period may be lower than onto

uncoated polyvinylchloride due to the difference in adsorption kinetics onto coated and uncoated polyvinylchloride. Furthermore, the physicochemical and thermodynamic properties of the adsorbed macromolecules may be similar to those of the surfactant coatings, thereby rendering the surface properties unchanged. The contributions of these two phenomena are under further investigation.

One of the major design criteria for biomaterials is the ability to resist or inhibit microbial adherence and, in so doing, the likelihood of medical-device-related infection will be decreased (Gorman & Jones 2002). Therefore, in this study the resistances of the various surfactant-coated biomaterials to microbial adherence (*S. aureus* and *Ps. aeruginosa*) were examined and compared with endotracheal tube polyvinylchloride. In addition, due to the importance of saliva on the surface properties of both microorganisms and biomaterials, the adherence assay was performed using uncoated polyvinylchloride and surfactant-coated polyvinylchloride and microorganisms that had been pre-treated with saliva. In addition, before inclusion in the adherence assay, the microorganisms were cultivated in a carbon-dioxide-enriched (5% v/v) atmosphere. It is believed that these conditions relate to the in-vivo scenario. Furthermore, as the microbial adherence assay that was used in this study utilised a sonication step to remove the adherent microorganisms from the various biomaterials, the effect of sonication was investigated on the surface properties of the materials under examination in this study. As may be observed, the advancing and receding contact angles of the various coated and uncoated samples of polyvinylchloride were statistically similar. Therefore, it may be concluded that the surfactant coatings remained intact and were unaffected by the sonication process. In addition, this provides evidence for the efficient bonding of the surfactant coatings onto medical-grade polyvinylchloride using a simple dip-coating process.

Importantly, adherence of both *Ps. aeruginosa* and *S. aureus* to the surfactant-coated biomaterials was significantly lower than to uncoated polyvinylchloride. Moreover, the magnitude of the reduction in adherence was substantial, frequently exceeding 90%. The nature of the coating influenced the subsequent microbial adherence at each exposure period (0.5, 1, 2, 4 and 8 h). In particular, the resistances of coatings composed of 0:100, 25:75 and 50:50 ratios of cholesterol to lecithin to the adherence of *S. aureus* were similar, whereas the resistance of the coating composed of the 75:25 ratio was lower (but greater than the resistance of uncoated polyvinylchloride). Similarly, the resistances of coatings composed of 0:100 and 25:75 ratios of cholesterol to lecithin to the adherence of *Ps. aeruginosa* were similar whereas the resistance of the coatings composed of 50:50 and 75:25 ratios were sequentially lower than, and once again superior to, that of uncoated polyvinylchloride. While the range of cholesterol–lecithin ratios was limited, this study has illustrated that the microbial anti-adherent effect was substantial, was due to lecithin and, in addition, was dependent on the nature of the microorganism. At this point it should be highlighted that the microbial anti-adherence properties of the various coatings examined were particularly impressive and were greater than previously reported for other polyvinylchloride-based biomaterials. For example, in a previous study the resistances of hexetidine-impregnated polyvinylchloride to the adherence of the isolates that were employed in this study were reported to be ca. 50% over a seven-day period (Jones et al 2002a). Therefore, it may be concluded that the coatings described in this study (particularly those composed of either 0:100 or 25:75 cholesterol–lecithin), if used as a coating for the lumen of endotracheal tubes, may reduce the incidence of ventilator-associated pneumonia.

Based on the results in this study, the mechanism of the microbial anti-adherent effect of the various coatings is unclear. Following treatment with saliva, the surface energies of the various surfactant-coated biomaterials and uncoated polyvinylchloride were statistically similar. As these biomaterials exhibited different resistances to the adherence of both *S. aureus* and *Ps. aeruginosa*, it may be concluded that the observed microbial anti-adherent effects were not directly related to differences in surface energetics. It may be suggested that, in light of the primary contribution of lecithin to the microbial anti-adherent properties, the observed resistance to microbial adherence may be due to steric hindrance associated with the hydrophilic regions of lecithin. There have been several reports that have ascribed the microbial anti-adherence effects of antimicrobial agents and hydrophilic polymers to their ability to sterically inhibit the interaction between the microbial cell and the epithelial cell or biomaterial substrate. For example, it was reported that treatment of either human buccal epithelial cells or *Candida albicans* in-vitro with cetylpyridinium chloride significantly reduced their subsequent interaction. After consideration of the effects of the antimicrobial agent on the surface properties of the microbial cell, the authors concluded that the adsorbed antimicrobial agent inhibited the microbial cell–epithelial cell interaction via steric interactions (Jones et al 1995a, b). Therefore, it is proposed that the microbial anti-adherent properties of the coatings under examination in this study are due, at least in part, to steric hindrance.

In conclusion, this study examined the physicochemical and microbial anti-adherent properties of endotracheal tube polyvinylchloride that had been coated with various ratios of cholesterol and lecithin (0:100; 25:75; 50:50 and 25:75). Importantly, it was shown that when compared with uncoated polyvinylchloride, the surfactant-coated biomaterials exhibited significantly decreased adherence of clinical isolates of *S. aureus* and *Ps. aeruginosa* for at least 8 h. The extent of the reductions in microbial adherence was substantial; that of the coating composed of either lecithin or 25% cholesterol and 75% lecithin frequently exceeding 90% when compared with the uncoated polyvinylchloride control. The surface energetics of the surfactant coatings were unaffected following sonication, indicating that the interaction of the surfactant coatings with the polyvinylchloride substrate was acceptable for clinical practice. In light of the substantial reductions in microbial adherence and durability, it may be concluded that the coatings composed of either lecithin or 25% cholesterol and 75% lecithin may reduce the incidence of

ventilator-associated pneumonia whenever employed as a luminal coating on endotracheal tubes. However, further studies are required to examine the in-vivo efficacy and acceptability.

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