Physicochemical Characterization of Hexetidine-Impregnated Endotracheal Tube Poly(Vinyl Chloride) and Resistance to Adherence of Respiratory Bacterial Pathogens

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Purpose. Ventilator-associated pneumonia is a frequent cause of mortality in intensive care patients. This study describes the physicochemical properties of hexetidine-impregnated poly(vinyl chloride) (PVC) endotracheal tube (ET) biomaterials and their resistance to microbial adherence (*Staphylococcus aureus* and *Pseudomonas aeruginosa*).

Methods. PVC emulsion was cured in the presence of hexetidine (0–20% w/w) and was characterized in terms of drug release, surface properties (i.e., microrugosity/contact angle), mechanical (tensile) properties, and resistance to microbial adherence.

Results. Under sink conditions, hexetidine release from PVC was diffusion-controlled. Increasing the concentration of hexetidine from 1% to 10% (w/w) (but not from 10% to 20% w/w) increased the subsequent rate of drug release. In general, increasing the concentration of hexetidine decreased both the tensile properties and hydrophobicity, yet increased PVC microrugosity. Following hexetidine release (21 days), the surface properties were similar to those of native PVC. The resistance of hexetidine-containing PVC (1% or 5%) to microbial adherence (following defined periods of drug release) was greater than that of native PVC and was constant over the examined period of hexetidine release.

Conclusions. ET PVC containing 1% (w/w) hexetidine offered an appropriate balance between suitable physicochemical properties and resistance to microbial adherence. This may offer an approach with which to reduce the incidence of ventilator-associated pneumonia.

KEY WORDS: PVC endotracheal tube; hexetidine; characterization; microbial adherence.

INTRODUCTION

The utilization of the endotracheal tube (ET) in the management of a patient receiving intensive care increases the risk (currently *circa* 20%) of nosocomial pneumonia (1–3). Intubation with the device overcomes the host defense factors in the respiratory tract such as the cough reflex and mucociliary escalator (4). Subsequent colonization of the device with potentially pathogenic microorganisms is followed by biofilm accretion, which predominates in the lumen of the tube (5,6). Aided by ventilatory gas flow, biofilm with encased microorganisms disseminates into an already compromised lower airway from where the onset of infection commences (3,7). As a result, urgent advances in the design of PVC ETs are required to resolve this problem.

The incorporation of an antimicrobial agent within the constituent polymer of a medical device is an accepted approach in engaging the problem of medical device-associated infection (8). This approach may reduce microbial adherence to the biomaterial by virtue of an antimicrobial surface, in addition to the release of drug into the surrounding medium in sufficient quantity to achieve microbial killing. The inclusion of an antimicrobial agent has resulted in beneficial outcomes in medical devices such as central venous catheters (9), bone cements (10), cerebrospinal fluid shunts (11), and continuous peritoneal dialysis catheters (12). More recently, the infection rates associated with two antimicrobial-impregnated central venous catheters, one containing minocycline and rifampicin and the other containing chlorhexidine and silver sulfadiazine, have been examined, with the authors concluding that catheters containing minocycline and rifampicin exhibited a lower rate of infection (13). Similarly Multanen *et al.* (14) described reduced microbial adherence to ofloxacin-blended, polylactone-coated, self-reinforced poly(l-lactide) ureteral stents and concluded that these drug-impregnated stents may reduce stent-associated infections However, the concept and evaluation of an ET tube incorporating an antimicrobial agent has yet to be fully explored.

In the design of antimicrobial-impregnated biomaterials, both the selection and the rate of release of the antimicrobial agent from the medical device are important. Ideally, the selected antimicrobial agent should possess broad-spectrum antimicrobial activity, a low incidence of bacterial resistance, and microbial antiadherence properties. The incidence of antibiotic resistance is well-documented, and, hence, an interest has emerged in the use of nonantibiotic, antimicrobial agents for incorporation within medical device biomaterials. In addition to their known broad-spectrum antimicrobial activity, nonantibiotic, antimicrobial agents (e.g., chlorhexidine, cetylpyridinium chloride, and cetrimide) have received particular attention for their *in vitro* microbial antiadherence properties (15). More recently, we have described the *ex vivo* antiadherence properties of the nonantibiotic antimicrobial agent hexetidine (16). In addition to its antimicrobial properties, the lipophilic nature of hexetidine renders it an excellent candidate for incorporation into hydrophobic biomaterials (e.g., ET poly(vinyl chloride) (PVC)).

Therefore, the aims of this study were, first, to examine the physicochemical properties and the release kinetics of hexetidine following impregnation within the PVC ET. As the incorporation of hexetidine into PVC may alter the mechanical and surface properties of the resultant biomaterial, and in light of the importance of the mechanical and surface properties of medical devices to their ultimate clinical performance (17,18), particular emphasis was placed on the characterization of these properties. In addition, a second aim of this study was to evaluate the resistance of the drug-containing biomaterial to adherence of the ET biofilm isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the requisite step in microbial colonization and, ultimately, in nosocomial pneumonia.

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MATERIALS AND METHODS

Chemicals

Sodium chloride, sodium dihydrogen orthophosphate, and disodium hydrogen orthophosphate were purchased from BDH Chemicals Ltd. (Poole, Dorset, United Kingdom). Medical grade PVC emulsion was purchased from Rusch Manufacturing Ltd. (Lurgan, Northern Ireland, United Kingdom). Warner Lambert (Dartford, Kent, United Kingdom) donated hexetidine. All other chemicals were of AnalaR quality and were purchased from Sigma-Aldrich (Poole, Dorset, United Kingdom).

Bacterial Isolates*

Clinical isolates of *S. aureus* and *P. aeruginosa*, which were derived from microbial biofilm present on the surface of PVC ETs that were retrieved from ventilated patients in the intensive care unit of Belfast City Hospital, were employed in this study (6,19). The isolates were maintained on beads (Protect Bacterial Preserve System, Technical Services Consultants Ltd., London, United Kingdom) and on agar in 10% glycerol at –70°C, and on Mueller Hinton agar slopes at 4°C, as previously described (6,19). Stationary-phase bacterial suspensions were grown by inoculating them in prewarmed Mueller Hinton broth and incubating them at 37°C for 16 h in 5% CO_2/95% air, which is representative of the atmospheric conditions within the oropharynx (19).

Collection of Saliva

Unstimulated saliva from six volunteers, none of whom were receiving medication, was collected, pooled, and clarified by centrifugation at 3,000 g for 10 min to remove any debris. The supernatant was diluted with an equal volume of sterile phosphate-buffered saline (PBS; pH 7.4) and was stored at 4°C in sealed containers prior to use. Saliva treatment of bacteria was performed as previously described (19).

Preparation of Hexetidine-Containing PVC Films

The preparation of PVC sheets devoid of hexetidine was performed as previously described (19). In brief, an aliquot of PVC emulsion (15 g) was located between two glass plates, separated by metallic spacers, and was heated at 160°C for 10 min. Similarly hexetidine-containing films were prepared by mixing known masses of hexetidine (1%, 5%, 10%, and 20% w/w) with PVC emulsion using a mechanical stirrer for 5 min. Following removal of entrapped air by sonication (5 min), a fixed mass of drug-loaded PVC emulsion was injected between glass plates and was cured at 160°C for 10 min, as before. To confirm the uniformity of drug distribution, discs were cut from randomly chosen areas of each film; hexetidine was extracted into acetonitrile and was quantified using high-performance liquid chromatography, as previously described (20).

In Vitro Release of Hexetidine

The release of hexetidine from hexetidine-containing PVC films was performed as previously described (21). Five sections (2 cm × 2 cm) of each biomaterial (containing 1, 5%, 10%, and 20% hexetidine w/w) were securely fixed to one end of separate microscope slides with vacuum grease. The sides of each section were covered carefully with vacuum grease, leaving one planar surface exposed for the release of hexetidine. Each section was immersed in a prewarmed (37°C) dissolution medium (50 ml) containing 1% (w/w) polysorbate (Tween) 20 in PBS (pH 7.4) and was placed in a shaking water bath at 37°C and 100 oscillations per minute. The inclusion of Tween 20 was required to ensure sink conditions. At selected time points, samples of dissolution medium (5 ml) were removed, and the mass of the hexetidine release was quantified using high-performance liquid chromatography, as previously described (20).

Microscopic Examination of Hexetidine-Loaded PVC

The surface roughness of PVC-impregnated hexetidine was investigated using atomic force microscopy (Burleigh Instruments, Victor, NY). The microrugosity of five PVC samples of each hexetidine concentration were studied prior to and 21 days after drug release from the polymer. The surface was imaged at a magnification of 10,000×, and the microrugosity was calculated as R_q (i.e., the root mean square of the vertical dimension of the surface). At least 50 measurements of surface roughness were made for each sample. All determinations were performed in quadruplicate using replicate samples (19).

Dynamic Contact Angle Measurement of Hexetidine-Loaded PVC Films

Sections of PVC incorporating hexetidine (1 cm × 1.5 cm) were suspended in a dissolution medium (PBS containing 1% w/w Tween 20) at 37°C for 3 weeks, with both planar surfaces available for the release of drug. The advancing and receding contact angles of freshly prepared hexetidine-loaded PVC films (1 cm × 1.5 cm) and those in which drug release had occurred were measured using a dynamic contact angle analyzer (DCA 312, ThermoCahn, Madison, Wisconsin) (19). The wetting medium was grade 1 reagent water, and an immersion rate of 150 μm/s was employed for all measurements. All determinations were performed at least in triplicate.

Determination of Mechanical Properties

Tensile testing of PVC films containing up to 10% (w/w) hexetidine was performed using a texture analyzer (Stable Micro Systems, Surrey, United Kingdom) with accompanying XT.RA Dimension software. The mechanical properties of films containing 20% (w/w) hexetidine were unsuitable for analysis. Sections of each biomaterial (7 cm × 1 cm) were vertically clamped, and the upper clamp was extended at a speed of 0.5 mm/s until sample fractured occurred. From the resultant stress-strain plot, the Young's modulus, ultimate tensile strength, and elongation at break were determined (22). All determinations were performed at least in quadruplicate.

Adherence of Bacteria to Hexetidine-Loaded PVC

Discs of PVC (6 mm in diameter) containing 0%, 1%, and 5% (w/w) hexetidine were fixed (in triplicate) to the base of sterile McCartney bottles using vacuum grease. A volume of prewarmed (37°C) dissolution medium (10 ml containing 1% Tween 20 in PBS at pH 7.4) then was added, and the bottles were incubated at 37°C in a shaking water bath (100

oscillations per minute) for time periods of 1 h, 6 h, 1 day, and 1 week. Sink conditions were maintained by the removal of a defined volume of dissolution medium on a daily basis and were replaced with fresh solution. After each time period, treatment of each biomaterial with saliva was performed by discarding the dissolution medium containing released hexetidine, washing with PBS, and adding diluted pooled saliva. The biomaterials were incubated with saliva for 30 m at 37°C, after which time saliva was decanted.

After each incubation time and each saliva treatment, the dissolution medium was removed from the bottles and replaced with a defined volume (10 ml) of stationary-phase, saliva-treated bacterial suspension (*circa* 1×10^7 colony-forming units/ml) in PBS. The bottles containing biomaterial discs and bacteria then were reincubated at 37°C for a period of 4 h. Following this period of incubation, discs were removed from the suspension and washed for 30 s in two 5-ml volumes of PBS, pH 7.4, to remove nonadherent microorganisms. Each disc then was added to a 10-ml volume of PBS and was sonicated for 5 min to dislodge adherent bacterial cells (19). Sonication was shown not to affect either microbial viability or morphology. After removal of the PVC discs, the number of viable bacteria was determined by serial dilution, as previously described (19).

Statistical Analysis

The effect of hexetidine concentration on the rate of drug release and on the mechanical properties (i.e., Young's modulus, elongation at break, and ultimate tensile strength) of PVC was statistically evaluated using a one-way analysis of variance. Conversely, the effects of the concentration of hexetidine and the time of release on the surface properties (i.e., advancing and receding contact angles, and microrugosity) of PVC and the number of adherent bacteria to each biomaterial were statistically examined using a two-way analysis of variance. *Post hoc* comparisons of the means of independent groups were performed using the Tukey honestly significant difference test. In all cases, $P < 0.05$ was accepted as denoting significance.

RESULTS

The release of hexetidine from drug-loaded PVC into 1% Tween 20 is illustrated in Fig. 1. To investigate the mechanism of drug release, the data generated from the release studies were fitted to the general release equation (21) using logarithmic transformations and least-squares regression analysis. For the formulations under investigation, release exponents (n) ranged from 0.48 to 0.53, which is indicative of diffusion-controlled release. The release of hexetidine was therefore proportional to the square root of time, the mean fractional release rates for films containing 1%, 5%, 10%, and 20% (w/w) hexetidine being 0.017 ± 0.001, 0.024 ± 0.002, 0.031 ± 0.002, and 0.026 ± 0.004 $h^{-1/2}$, respectively. With the exception of 20% (w/w) hexetidine, increased drug loading significantly increased the subsequent rate of drug release.

The mechanical properties of PVC, both devoid of and containing hexetidine (1–10% w/w) are presented in Table 1. As the incorporated concentration of hexetidine increased from 0% to 1% and from 1% to 5% (w/w) hexetidine, there were significant reductions in both the ultimate tensile strength and the percentage of elongation at break, however, the ultimate tensile strengths and elongation properties of 5% and 10% drug-loaded PVC were significantly similar. Increasing the concentration of hexetidine from 0% to 1% did not significantly affect the Young's modulus, however, the Young's modulus of PVC containing 5% hexetidine was significantly lower than comparator films there were either devoid of or contained 1% hexetidine. No significant differences were observed in the mechanical properties (i.e., ultimate tensile strength, percentage elongation at break, and Young's modulus) of PVC containing either 5% or 10% hexetidine.

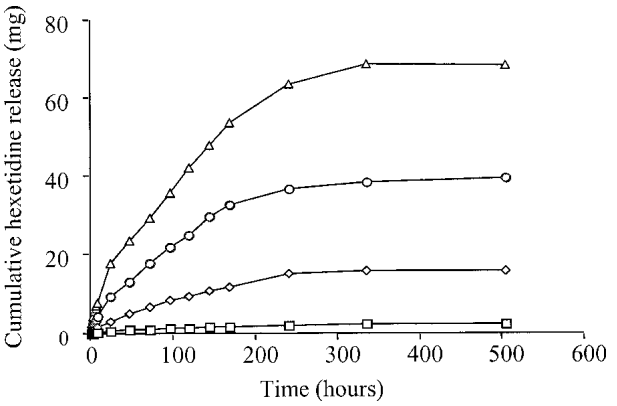

Fig. 1. The effect of loading on the release properties of hexetidine from a PVC matrix (mean ± SD cumulative mass of the hexetidine released; the SD is smaller than symbols). Key for concentrations: 1% w/w (squares); 5% w/w (diamonds); 10% w/w (circles); and 20% w/w (triangles).

The microrugosity (surface roughness) of hexetidine-impregnated PVC, both before and after drug release, is presented in Table 2. As may be observed, the inclusion of hexetidine in PVC significantly increased the microrugosity of the polymeric films for each drug loading. The microrugosities of 1%, 5%, and 10% (w/w) hexetidine-loaded films were statistically similar; however, films containing 20% (w/w) hexetidine possessed the greatest microrugosity. Comparison of surface roughness measurements of polymer sections before and after drug release revealed that the surfaces were significantly smoother after 21 days of hexetidine release and approached the microrugosity of PVC films that were devoid of hexetidine. After this period of drug release, the surface microrugosities of films originally containing 1%, 5%, and 10% (w/w) hexetidine were statistically similar, whereas films containing 20% (w/w) hexetidine once more exhibited significantly greater microrugosity.

Table I. The Effect of Hexetidine Incorporation (% w/w) on the Tensile Properties of PVC[a]

Hexetidine concentration (% w/w)	Mechanical property		
	Ultimate tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
0	2.89 ± 0.09	285.93 ± 10.28	1.12 ± 0.05
1	1.28 ± 0.08	114.71 ± 5.28	1.15 ± 0.08
5	0.85 ± 0.08	39.42 ± 1.82	0.75 ± 0.01
10	0.90 ± 0.04	40.54 ± 1.93	0.73 ± 0.02

[a] Values given as mean ± SD.

Table II. The Surface Roughness of Hexetidine-Loaded PVC before and after Release of Drug[a]

Hexitidine concentration in PVC (% w/w)	Surface roughness (nm)	
	Before release	After release
0	55.72 ± 12.86	
1	136.95 ± 14.01	70.47 ± 11.88
5	136.19 ± 26.60	82.42 ± 10.27
10	141.66 ± 35.03	78.31 ± 5.64
20	267.52 ± 73.99	95.97 ± 6.19

[a] Values given as mean ± SD.

Table 3 presents the advancing and receding contact angles of PVC films containing a range of concentrations of hexetidine (0–20% w/w) both prior to and 21 days after drug release. As may be observed, the surface of PVC films were hydrophobic (i.e., they exhibited a high advancing contact angle). The incorporation of hexetidine significantly decreased the surface hydrophobicity of PVC in a concentration-dependent fashion. Thus, increasing the concentrations from 0% to 1% to 5% to 10% (w/w) significantly decreased the advancing contact angle of PVC. However, no further significant decreases in hydrophobicity were observed as the concentration of hexetidine in PVC was increased from 10% to 20% (w/w). The incorporation of hexetidine into PVC significantly decreased the receding contact angle of this biomaterial. However, the receding contact angles of PVC containing 1%, 5%, 10%, and 20% hexetidine were similar. Following drug release for 21 days under sink conditions, the advancing and receding contact angles of PVC containing hexetidine (1%, 5%, 10%, and 20% w/w) were significantly greater than those observed prior to drug release and, indeed, approached the advancing contact angle of PVC devoid of hexetidine.

In this study, viable bacterial adherence to hexetidine-loaded PVC has been expressed as the percentage of viable bacteria adherent to control discs composed of PVC alone. The adherence of viable *S. aureus* to PVC incorporating 1% and 5% (w/w) hexetidine, treated with pooled saliva, is presented in Table 4. Both immediately after manufacture (0 h) and following release periods of 1 h, 6 h, 24 h, and 7 days, the adherence of *S. aureus* to saliva-treated, hexetidine-containing PVC was significantly lower than to PVC alone. Furthermore, the resistance of PVC containing 5% (w/w) hexetidine to the adherence of *S. aureus* was significantly greater than the counterpart containing 1% (w/w) of this antimicrobial agent. Interestingly, the resistance of the PVC that had been impregnated with hexetidine (either 1% or 5% w/w) to microbial colonization was similar at each of the release periods.

Table IV. The Effect of Time of Release of Hexetidine from Hexetidine-Impregnated ET PVC on the Subsequent Adherence of *S. aureus*[a]

Duration of hexetidine release	Viable bacterial adherence	
	1% w/w hexetidine	5% w/w hexetidine
0 h	70.25 ± 5.95	27.98 ± 3.58
1 h	75.69 ± 4.59	28.32 ± 1.21
6 h	69.57 ± 3.56	28.65 ± 1.01
24 h	68.57 ± 5.54	20.58 ± 3.98
7 days	70.28 ± 5.88	24.69 ± 3.25

[a] Values given as the mean ± SD percentage (%) of adherence to the PVC control, to hexetidine-impregnated PVC.

Table 5 describes the adherence of viable *P. aeruginosa* to saliva-treated hexetidine-impregnated PVC. Both freshly prepared hexetidine-containing PVC and, additionally, PVC from which hexetidine had been allowed to release prior to inclusion in the adherence assay exhibited significantly reduced adherence of *P. aeruginosa* in comparison to PVC devoid of hexetidine (control). Once more, PVC containing 5% (w/w) hexetidine exhibited a greater resistance to colonization with *P. aeruginosa* than PVC containing 1% (w/w) of this antimicrobial agent, and, in addition, the resistance of the hexetidine-impregnated PVC (either 1 or 5% w/w) to microbial colonization was similar at each of the release periods.

DISCUSSION

The ET is clinically used in the mechanical ventilation of patients receiving intensive care. However, the introduction of medical devices into the oropharynx is commonly followed by the adherence of microorganisms to the device, a step that is accepted to represent the initial stage in the process of ET colonization and biofilm formation (1,2). The importance of this process may not be overlooked, and, indeed, in a recent study it was confirmed using molecular techniques in which the microorganisms within the microbial biofilm were involved in the pathogenesis of ventilator-associated pneumonia (3). The resistance to antibiotics afforded to the microorganisms within the biofilm renders conventional antimicrobial chemotherapy relatively ineffective, and frequently device removal is necessitated. However, one alternative approach to

Table III. Advancing and Receding Contact Angle of Hexetidine-Loaded PVC before and after Release of Drug[a]

Hexetidine loading (% w/w)	Before release		After release	
	Advancing contact angle (°)	Receding contact angle (°)	Advancing contact angle (°)	Receding contact angle (°)
0	92.64 ± 0.82	69.17 ± 0.43	—	—
1	86.11 ± 1.04	65.77 ± 2.23	89.16 ± 1.04	69.93 ± 1.08
5	81.08 ± 1.00	66.85 ± 2.18	89.63 ± 0.38	69.73 ± 2.05
10	77.21 ± 1.14	65.78 ± 2.11	86.96 ± 0.20	65.80 ± 1.13
20	77.71 ± 0.51	63.05 ± 4.01	82.65 ± 1.15	68.79 ± 0.01

[a] Values given as mean ± SD.

Table V. The Effect of Time of Release of Hexetidine from Hexetidine-Impregnated ET PVC on the Subsequent Adherence of *P. aeruginosa*[a]

Duration of hexetidine release	Viable bacterial adherence	
	1% w/w hexetidine	5% w/w hexetidine
0 h	51.61 ± 3.99	43.31 ± 3.91
1 h	57.66 ± 6.15	46.69 ± 5.01
6 h	48.01 ± 2.63	48.33 ± 3.57
24 h	56.69 ± 6.24	46.73 ± 4.09
7 days	51.28 ± 2.99	47.08 ± 4.38

[a] Values given as the mean ± SD percentage (%) of adherence to the PVC control, to hexetidine-impregnated PVC.

obviate this problem involves the incorporation of antimicrobial agents into the ET tube biomaterial (8–12).

In the development of a drug-impregnated PVC biomaterial, one of the most important decisions regards the choice of the antimicrobial agent. There are a number of factors that influence this choice. First, the antimicrobial agent should possess a suitable spectrum of antimicrobial activity. Second, the impregnated antimicrobial agent should ideally be soluble within the biomaterial to ensure that the surface microrugosity is not adversely affected during the release process. The deleterious effects of heterogeneous matrix systems on the surface topography of polymers have been reported (21). Third, the release rate of the antimicrobial agent should be sufficient to provide the required antimicrobial effect. Finally, the mechanical and surface properties of the biomaterial should not be compromised as a result of the presence of the incorporated therapeutic agent, and, accordingly, the functional demands of the device should be maintained. The antimicrobial agent employed in the present investigation, hexetidine, was regarded as a suitable candidate for such a purpose on the basis of its broad spectrum of antimicrobial activity, its nonprovocation of resistance, and its low toxicity profile (23). Following incorporation into PVC, the lipophilicity and low melting point of hexetidine ensured that, at the majority of concentrations examined (i.e., 1%, 5%, and 10%) hexetidine was present as a molecular solution within the biomaterial. The mechanism of release from these systems was diffusion-controlled, as defined by the statistical similarities of the calculated release exponents to 0.5 (21). Increasing the concentration of hexetidine in these systems from 1% to 10% (w/w) increased the subsequent rate of release, as may be predicted from diffusion theory. Therefore, a range of hexetidine release rates was achieved by the manipulation of the concentration of hexetidine within the biomaterial. Interestingly, the incorporation of 20% (w/w) hexetidine did not significantly increase the release rate when compared to systems containing 10% (w/w) of this therapeutic agent. This disparity may be accredited to the deleterious effect of this higher loading on the mechanical properties of PVC and the insolubility of hexetidine within the PVC matrix.

Under US Food and Drug Administration regulations, the ET tube is categorized as a class III critical device, which is defined as "a device intended for surgical implant into the body or to support or sustain life, and whose failure to perform when properly used ... can reasonably be expected to result in a significant injury to the user" (24). Therefore, in light of the importance of the mechanical properties of the ET tube in the maintenance of air flow into and out the respiratory tract, it is important to characterize the effect of the incorporated antimicrobial agent on these properties. For this reason, the tensile properties of the candidate biomaterials were examined. Tensile analysis of the hexetidine-loaded biomaterials revealed significant differences in the various mechanical properties when compared to the PVC that was devoid of this therapeutic agent. In particular, the inclusion of hexetidine produced materials of lower tensile strength, percentage elongation at break, and Young's modulus. These results, and the observed solubility of hexetidine into PVC, would suggest that the interaction of hexetidine with PVC interfered with the interaction between adjacent chains of PVC, or alternatively, interfered with the initial curing reaction. In this fashion, the ability of the material to resist deformation following exposure to an applied (tensile) stress was reduced. Interestingly, the incorporation of 20% (w/w) hexetidine rendered PVC unsuitable for tensile analysis due to a loss of mechanical structure. These effects correlate with the unusual release properties of this biomaterial. Based on these observations, it may be concluded that the concentration of hexetidine should be minimized to ensure that the mechanical properties of PVC are not overtly compromised.

The importance of the effect of the surface properties of biomaterials on the resultant biologic performance has been recorded in several studies. For example, a correlation between the microrugosity of peritoneal dialysis catheters and the adherence of *Staphylococcus epidermidis* was demonstrated using confocal laser scanning microscopy and atomic force microscopy, with greater adherence being observed in catheters with increased microrugosity (25,26). Similarly, the contribution of biomaterial surface energetics to the microbial adherence process has been recognized (27). In light of the importance of biomaterial surface properties, the effects of hexetidine incorporation on the contact angle and microrugosity of PVC were investigated. The measurement of these properties revealed significant alterations to the nature of the biomaterial surface, which resulted from the incorporation of hexetidine. The general increase in surface roughness and the increased hydrophilicity associated with increasing concentrations of incorporated hexetidine suggests the presence of hexetidine at the surface of PVC. Interestingly, following the release of hexetidine for 21 days under sink conditions, a period over which the concentration of hexetidine within the PVC was depleted, the surface properties of this biomaterial were similar to native PVC. This illustrates both the direct dependence of the surface properties of PVC on the presence of hexetidine and, in addition, the reversible nature of these effects following drug release. The effects of hexetidine on the surface roughness suggest the accumulation of this therapeutic agent at the PVC/air interface.

The therapy of medical device-associated infections is frustrated by microbial resistance to antibiotics, and, consequently, the removal of the device is often required. However, the alternative approach of incorporating antimicrobial agents into the constituent biomaterials of medical devices attempts to resolve this issue before it becomes a problem. The concept has been employed successfully in a wide variety of materials with reports of reduced microbial adhesion to, for example, tobramycin-impregnated poly(methylmethacrylate) bone cement (10), rifampicin-loaded silicone cerebrospinal

fluid shunts (11), polyurethane central venous catheters containing chlorhexidine and silver sulphadiazine (9), penicillin-releasing silicone continuous ambulatory peritoneal dialysis (CAPD) catheters (12), and methacrylate dental resin incorporating antimicrobial agents (28). As yet absent from the literature, the incorporation of a suitable antimicrobial agent into PVC prior to the manufacture of ET tubes may hold benefits in combating the second-most common hospital-acquired infection, nosocomial pneumonia. Increasing the concentration of hexetidine in PVC was observed to beneficially increase the resultant rates of drug release and, hence, to increase the resistance of the material to microbial adherence and colonization. However, this benefit was offset by alterations to the mechanical properties of the biomaterial. The effects of incorporation of 1% (w/w) hexetidine on the mechanical properties of PVC were less in comparison to PVC containing higher concentrations of this agent. Therefore, as a compromise, the resistance of PVC in which 1% or 5% (w/w) hexetidine had been incorporated to microbial adherence was examined. The microbial adherence assay was conducted using saliva-coated bacteria and biomaterials, as the presence of saliva has been previously shown by us to affect the process of microbial adherence to biomaterials due to the formation of a conditioning film on these substrates (19). Furthermore, the bacterial isolates were grown in a carbon dioxide-enriched atmosphere, as this is representative of physiologic conditions and has been reported to alter the chemical composition of the microbial surface, the microbial adherence to biomaterials, and the susceptibilities of microorganisms to antimicrobial agents (19). Importantly, in this study it was shown that following different periods of release under sink conditions (up to 7 days) and subsequent incubation of the hexetidine-containing biomaterials in a bacterial suspension, there were significant reductions in the number of adherent viable bacteria to the various biomaterials. Furthermore, this effect was concentration-dependent, with lower observed viable adherence of both *S. aureus* and *P. aeruginosa* to PVC that had been impregnated with 5% hexetidine. The solubility of hexetidine in the medium in which the microbial adherence assay was performed is low.

Therefore, it may be postulated that the release of hexetidine during the period of the microbial adherence was controlled both by the diffusion of hexetidine through the PVC and by the removal of this agent from the interface between the biomaterial and the aqueous medium. Two important observations emerged from this study that offered an insight into the antimicrobial properties of these materials. First, the time of release of hexetidine (prior to inclusion in the microbial adherence assay) did not significantly affect subsequent microbial adherence to the candidate biomaterials. Conversely, the adherence of the microorganisms to PVC containing 5% (w/w) hexetidine was consistently lower than to the comparator material containing 1% (w/w) of this agent. In diffusion-controlled release, the diffusion boundary of the therapeutic agent recedes and, accordingly, the rate of release decreases as a function of time (29). Therefore, prior to the introduction of the biomaterials into the aqueous medium of the microbial adherence assay, it may be concluded that, in accordance with diffusion theory, the mass of hexetidine at the surface of the PVC decreased as a function of time and increased as a function of initial drug loading. Differences in the mass of hexetidine present at the surface of PVC containing either 1% or 5% (w/w) of this antimicrobial agent are supported by the differences in the advancing contact angles of the two materials. Following introduction into the microbial adherence assay, a mass of drug will be released, however, in light of the poor aqueous solubility of this compound and the known minimum inhibitory concentrations of hexetidine against *S. aureus* and *P. aeruginosa* (1.95 and 250 μg/ml, respectively) (30), it is suggested that the microbial antiadherence effects presented in Tables 4 and 5 are primarily due to the presence of hexetidine at the interface between the aqueous medium and the biomaterial. However, the contribution of the bactericidal properties of hexetidine that has been released into the aqueous medium to the overall antimicrobial properties of the drug-impregnated PVC biomaterials cannot be dismissed. In a previous report (16), the microbial antiadherence properties of hexetidine were described, and, therefore, the observed resistance of the hexetidine-impregnated biomaterials to microbial adherence may be partly due to these known properties. Regardless of the exact mechanism of the antiadherence, it is important to highlight the greater ability of the hexetidine-impregnated PVC biomaterials to resist microbial colonization in comparison to PVC devoid of this therapeutic agent.

Interestingly, the incorporation of hexetidine was observed to significantly increase the microrugosity of PVC, a phenomenon that has been reported to increase microbial adherence to biomaterials (26,27). Based on this parameter alone, it would be expected that the adherence to hexetidine-impregnated PVC would increase as the concentration of the incorporated drug increased. The results from this study would indicate that the antimicrobial effects of hexetidine were sufficiently large to negate the possible problems associated with increased surface microrugosity. This serves to highlight the complex interplay of physicochemical parameters that dictate the process of microbial adherence to biomaterials.

In conclusion, this study has described the formulation of hexetidine-impregnated PVC as a candidate ET tube biomaterial and, in addition, its physicochemical and biologic properties. In particular, the candidate biomaterials were examined with respect to their tensile, surface, and drug-release properties, and, additionally, with respect to their resistance to the adherence of clinical isolates of *S. aureus* and *P. aeruginosa* that are derived from the microbial biofilm present on the surface of PVC ETs. Significantly, this study has demonstrated that, in light of their comparative resistance to microbial adherence, the use of PVC biomaterials containing impregnated hexetidine may reduce the incidence of ventilator-associated pneumonia. In these systems, the concentration of hexetidine should be carefully selected to ensure that the mechanical properties of the biomaterial are suitable for clinical use. However, further investigations are required to confirm the biocompatibility of these systems and their *in vivo* efficacy.

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