Novel Self-Disinfecting Surface

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ABSTRACT: The colonization of medical devices by micro-organisms is an ongoing problem, particularly as many strains of bacteria are becoming resistant to antibiotics. One method by which this could be addressed is the production of a surface that will kill bacteria on contact without releasing the disinfecting agent into solution. Self-disinfecting surfaces were prepared in which a quaternary ammonium salt was covalently bound onto a polyethylene film by a stable linkage. To achieve this, a low-density polyethylene film was treated with glow discharge followed by the graft polymerization of acrylic acid. The acid chloride derivative was then prepared. α,ω-Dibromoalkanes of various chain lengths were reacted with potassium phthalimide to form an *N*-alkylphthalimido group, and this was followed by an excess of piperidine to produce a tertiary amino group at the opposite end of the alkyl chain. The hydrolysis of the phthalimido group produced a primary amino function that was coupled to the grafted poly(acryl chloride). A reaction with octadecyl bromide resulted in the production of a quaternary ammonium salt. The amide linkage by which the quaternary ammonium salt was attached to the polymer was stable, and no release of the disinfecting moiety took place in solution. Soluble compounds containing a similar quaternary ammonium function were prepared through the reaction of the primary amine group with acetyl chloride rather than the polymeric acid chloride to form substituted *N*-acetamides. In a microbiological pilot study, both the polymer-bound and soluble quaternary ammonium salts were effective against suspensions of *Staphylococcus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae*. The results show that a self-disinfecting surface can be produced in which the quaternary ammonium function is an effective bacteriocide that remains bound to the polymer surface. In addition, the materials were toxic to a mammalian cell line (Chinese hamster ovary cells). J Appl Polym Sci 100: 381–389, 2006

Key words: biological applications of polymers; graft copolymers; polyethylene (PE); surfaces

INTRODUCTION

Vascular catheters such as Hickman lines are among the most frequently used medical devices, and they find considerable application in the treatment of chronic and critically ill patients.[1] One of the leading complications of such devices is catheter-related sepsis, in which colonization by micro-organisms such as coagulase-negative staphylococci can result in life-threatening conditions such as septicemia. Indeed, vascular catheters are a major source of nosocomial sepsis and contribute to the majority of cases of nosocomial cases of septicemia due to micro-organisms such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida* species.[1]

Several approaches have been described in previous investigations[1–9] that have been aimed at combating this added complication of infection associated with long-term indwelling vascular catheters. These are described in a separate publication.[10]

These methods have the disadvantage that the drug may be lost from the polymer. Loss can occur from the site of topical administration, by diffusion or ion exchange from surface coatings, or by diffusion from the polymer matrix; this places a finite life span over which the material can retain its disinfectant properties.

Our approach to this problem is to provide a polymer surface to which an antimicrobial agent such as a quaternary ammonium salt is covalently bound. Quaternary ammonium salts are well-known cationic molecules that are able to disrupt the bacterial cell membrane, causing the contents to leak out and resulting in the death of the cell.[11,12] A surface produced in this way should remain self-disinfecting over the required lifetime of the device and thus prevent colonization of the catheter or other medical device by micro-organisms and subsequent infection of the patient during the treatment period.

Another advantage of choosing a disinfectant such as a quaternary ammonium salt is the emergence of multiantibiotic-resistant bacteria associated with catheters, vascular grafts, and prosthetic-joint-related infections.

To produce such a material, the polymer onto which the quaternary ammonium salt will be attached must either contain functional groups or must be capable of

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being functionalized. A convenient way of introducing functional groups onto an inert polymer such as polyethylene (PE) is the graft polymerization of a suitable monomer containing functional groups such as acrylic acid or acrylamide and related compounds.[13–22] Some success has also been achieved with carboxy-substituted carbene and nitrene insertion reactions in producing functional groups directly onto the polymer surface.[23]

Soluble polymers based on poly(glycidyl methacrylate) and containing bound quaternary ammonium and phosphonium groups have recently been reported in the literature.[24] Such polymers are effective against a range of Gram-positive and Gram-negative organisms. These findings suggest that our own approach of using a quaternary ammonium compound covalently bound onto an insoluble support may well provide an effective self-disinfecting surface.

To achieve our objective, we grafted acrylic acid onto a PE film after a prior treatment with glow discharge to produce free radicals and peroxides on the surface. After the completion of the graft polymerization process, which was confirmed by IR spectroscopy and quantified by the reaction of the carboxyl groups with a cationic dye, the grafted poly(acrylic acid) was reacted with phosphorus pentachloride to form the reactive acid chloride derivative of the carboxyl side chains. The acid chloride so formed was then allowed to react with a bifunctional molecule containing an amino group at one end and a tertiary amine or quaternary ammonium function at the other separated by a straight carbon chain of 4–10 methylene groups. Hence, the tertiary amine or quaternary ammonium salt was coupled to the grafted polymer via an amide linkage formed between the acid chloride and primary amino groups. The tertiary amine function was then further reacted with an alkyl halide of varying chain length to form the corresponding quaternary ammonium salt.

Materials produced in this way all were proved to have disinfecting properties. The quaternary ammonium function, coupled to the polymer backbone by an amide linkage, remained covalently attached over the test period, thus providing potentially long-term bactericidal properties.

With the reaction schemes described later, a series of quaternary ammonium functions immobilized onto the PE surface were prepared. To test the efficacy of the quaternary ammonium functions themselves, a series of compounds was prepared as their substituted acetamides through the reaction of the bifunctional moieties with acetyl chloride to form soluble derivatives.

In a preliminary microbiological survey, both immobilized and soluble quaternary ammonium compounds were tested against a series of Gram-positive and Gram-negative micro-organisms for their antibacterial effect. In addition, the materials were exposed to a variety of cell lines to assess any potential cytotoxicity.

EXPERIMENTAL

All chemicals were purchased from Aldrich Chemicals, Ltd. (Poole, United Kingdom), and were used without further purification except for acrylic acid, which was purified by vacuum distillation before use. All solvents were analar-grade.

Linear low-density PE film was kindly supplied by BP Chemicals (Grangemouth, UK).

Ultraviolet spectra were recorded on a PerkinElmer (Beaconsfield, UK) Lambda 3 ultraviolet–visible spectrophotometer. IR spectra were recorded on a Nicolet (Warwick, UK) 420 Impact Fourier transform infrared (FTIR) spectrometer, and the compounds were examined either as grafts on PE film or as solids in KBr pellets. ^{1}H- and ^{13}C-NMR were recorded at 90 Mz with a JEOL (Welwyn Garden City, UK) EX90 spectrometer with tetramethylsilane as a standard.

All cell culture materials were obtained from Life Technologies (Paisley, UK). The Chinese hamster ovary cell line DG44 was a generous gift from Dr. Larry Chasin (Columbia University, New York, NY).

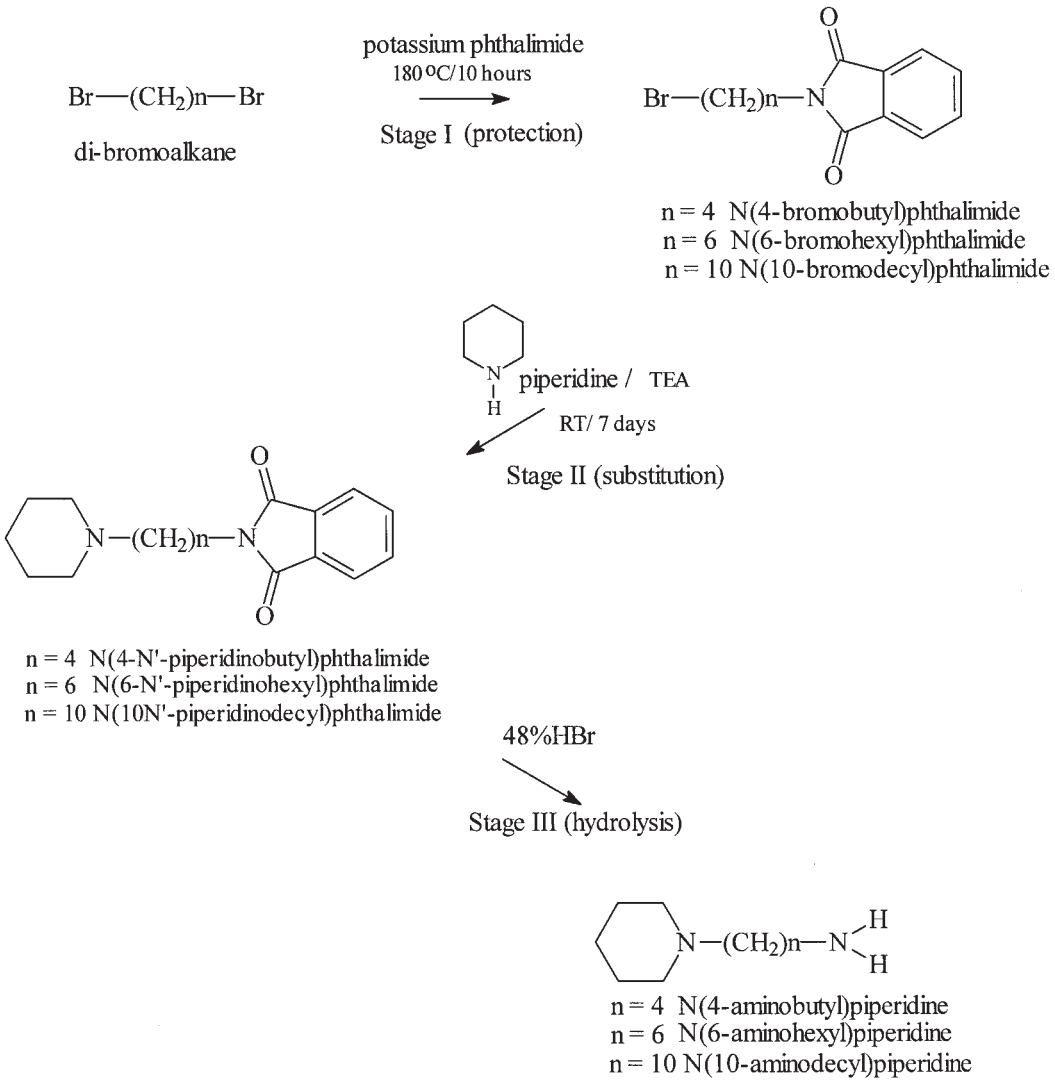
The graft polymerization of acrylic acid onto PE, the determination of the graft density of carboxyl groups on PE film, and acid chloride formation of grafted carboxyl groups on PE were all carried out according to a methodology previously described.[10]

Synthesis of a series of aminoalkylpiperidines

A series of compounds were prepared that contained both primary and tertiary amino functional groups. Once formed, the compounds could either be coupled to the PE film via an amide linkage or alternatively be used in the preparation of a number of model soluble amides. Three compounds were prepared with dibromoalkanes of different chain lengths (4, 6, or 10 carbon atoms). A synthesis was carried out in three stages, as shown in Scheme 1, essentially according to the method described by Riggio et al.[25]

In stage I, the dibromide was protected at one end. Potassium phthalimide was reacted with the dibromoalkane in a 1:3 molar ratio at 180°C for 10 h. The resultant mixture was distilled in vacuo, and three fractions were collected. The first fraction was unreacted dibromoalkane, the second was a mixture of the reactants and a byproduct, and the third fraction was the desired product, which was subsequently recrystallized from ethanol.

Stage II involved a substitution of the remaining bromide with the cyclic amine, piperidine The *N*-bromoalkylphthalimide product (II) from stage I (0.05 mol) was added to benzene (200 mL) containing piperidine (0.1 mol) and triethylamine (0.05 mol), and the reaction was stirred for 7 days. After the reaction,



Scheme 1 Preparation of a series of aminoalkylpiperidine compounds.

triethylamine hydrobromide was removed by filtration. The solvent was then removed *in vacuo* (40°C), and the residue was triturated with diethyl ether to produce a solid, *N*-(4-*N'*-piperidinoalkyl)phthalamide (III).

In stage III, the phthalyl group was then removed by the refluxing of the product (0.02 mol) in 48% HBr (25 mL). The precipitate of phthalic acid was removed by filtration, and the pH of the supernatant was adjusted to 9 with sodium bicarbonate. The resulting solution was then extracted with chloroform, and the organic layer was isolated and washed with water. The organic layer was dried (calcium chloride), and the solvent was removed by rotary evaporation to yield *N*-(aminoalkyl)piperidine (IV).

The structures of the aforementioned products from stages I–III were confirmed by IR and NMR spectroscopy. The following assignments are proposed as an example of the NMR data obtained for one of the compounds [*N*-(4-aminobutyl)piperidine] from stage III (n = 4). Similar corresponding data were obtained for the other compounds that confirmed their structures (not shown as each compound only differed by one methylene group).

^{1}H-NMR ($CDCl_3$, δ, ppm): 1.3 (m, 2H, NH_2CH_2-CH_2CH_2), 1.5 (broad, m, 6H, $NH_2CH_2CH_2$, N-ring $CH_2CH_2CH_2$), 2.1 (broad, s, NH_2), 2.3 (m, 2H, $NH_2CH_2CH_2CH_2CH_2N$), 2.65 (m, 2H, CH_2NH_2), 3.3 (t, 2H, N-ring CH_2). ^{13}C-NMR ($CHCl_3$, δ, ppm): 23.9 ($NH_2CH_2CH_2$), 25.2 (N-ring CH_2CH_2), 25.2 (N-ring $CH_2CH_2CH_2$), 28.4 ($NH_2CH_2CH_2CH_2$), 40 (NH_2CH_2), 54 (CH_2N-ring), 58 (N-ring CH_2).

IR spectroscopy shows absorption bands due to C—H stretching at ~2900 and 1460 cm^{-1}, C—N at 1395 cm^{-1}, and N—H at 3400 and 1630 cm^{-1}. Complete hydrolysis of the phthalyl group is confirmed by the absence of any carbonyl stretches in the 1715- and 1774-cm^{-1} region and in NMR by the absence of the carbonyl carbons and the aromatic carbons and protons.

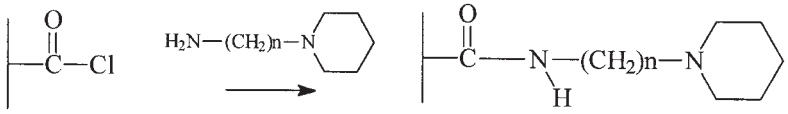


Figure 1 Immobilization of the tertiary amine to the acid chloride film (n = 4, 6, or 10).

The compounds from stage III were then either coupled to the acid chloride film or alternatively used for the preparation of the model soluble amides.

Covalent attachment of an aminoalkylpiperidine to the grafted PE film

The coupling of the aminoalkylpiperidine compounds to the film was via an amide linkage. The piperidine derivative (0.5 g) was dissolved in dry pyridine (30 mL) and allowed to react with the acid chloride film for 24 h at the ambient temperature, after which the films were removed, washed in ether, dried, and analyzed by FTIR spectroscopy (Fig. 1).

Quaternization of the tertiary amine on the PE film

A quaternary ammonium salt could then be formed from the tertiary amine prepared previously through the refluxing of the film with bromooctadecane (1 g) in ether (30 mL) for 7 days (see Fig. 2). The films were subsequently washed thoroughly in diethyl ether, dried, and stored for microbiological testing.

The differences that can observed by IR spectroscopy between the tertiary amine and the quaternary ammonium salt are very small. Confirmation of quaternization was indicated by the presence of bromide counterions, which were precipitated as silver bromide upon a reaction with a silver nitrate solution.

Stability of the amide linkage on the PE film

The stability of the amide linkage was investigated with a colorimetric assay in which free quaternary ammonium salts liberated into solution were assessed with a modification of a standard literature procedure.[26] The quaternary ammonium film was incubated at the ambient temperature in a phosphate buffer (pH 7; 10 mL) for various times (5 min, 30 min, 60 min, 4 h, and 24 h). The solution was analyzed for the presence of the quaternary ammonium salt with the dye bromophenol blue as follows. After the removal of the film from the incubation mixture, a solution of 10% sodium carbonate (2 mL) and 1 mL of a bromophenol aqueous solution (4 g/L) was added. The mixture was then extracted with dichloromethane (10 mL), and the absorbance of the organic layer was determined at 604 nm. The amount of quaternary ammonium released was determined from a standard curve of bromophenol blue versus the absorbance at 604 nm. The films were then analyzed by FTIR spectroscopy. The assay procedure was standardized with cetyl pyridinium bromide.

Preparation of soluble quaternized amide ammonium compounds

The aminoalkylpiperidine derivatives (IV) from stage III of Scheme 1 (0.02 mol) were reacted with acetic anhydride (0.1 mol) and one drop of concentrated sulfuric acid in a boiling water bath for 10 min. The reaction mixture was then poured onto ice-cold water (5 mL), and the pH was adjusted to 9.0 with saturated sodium bicarbonate. After extraction with chloroform, the solvent was removed by rotary evaporation, and this afforded a range of soluble amide ammonium compounds. The amides (0.01 mol) were then further reacted with 1-bromooctadecane (0.1 mol) in chloroform (75 mL) to form the quaternary salts (see Fig. 3).

The structures were subsequently analyzed with spectroscopy. The FTIR spectra showed a carbonyl stretch for the amide group at 1660 cm^{-1} as expected. The following assignments are proposed as an example of the main NMR data obtained for one of the compounds (n = 6). Again, full data for the remaining compounds are not shown as each only differed by one methylene group.

^{1}H-NMR ($CDCl_3$, δ, ppm): 0.9 [t, 3H, $CH_3(CH_2)_{17}N$], 1.3 [m, broad, $CH_3(CH_2)_{15}$, $NHCH_2CH_2$], 1.5 (m, $NHCH_2$ and N^+ ring $CH_2CH_2CH_2$), 1.8 [m, 2H, $CH_3(CH_2)_{15}CH_2$], 2.0 (s, 3H, $CH_3C{=}O$), 2.7 [m, $NH(CH_2)_2CH_2CH_2CH_2$], 3.2–3.4 [broad, m, $NH(CH_2)_5$-

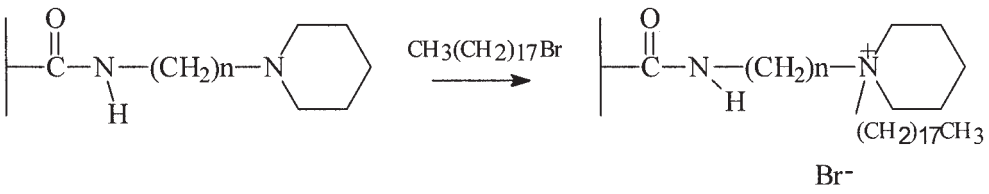


Figure 2 Quaternization of the tertiary amine on the film (n = 4, 6, or 10) by bromooctadecane.

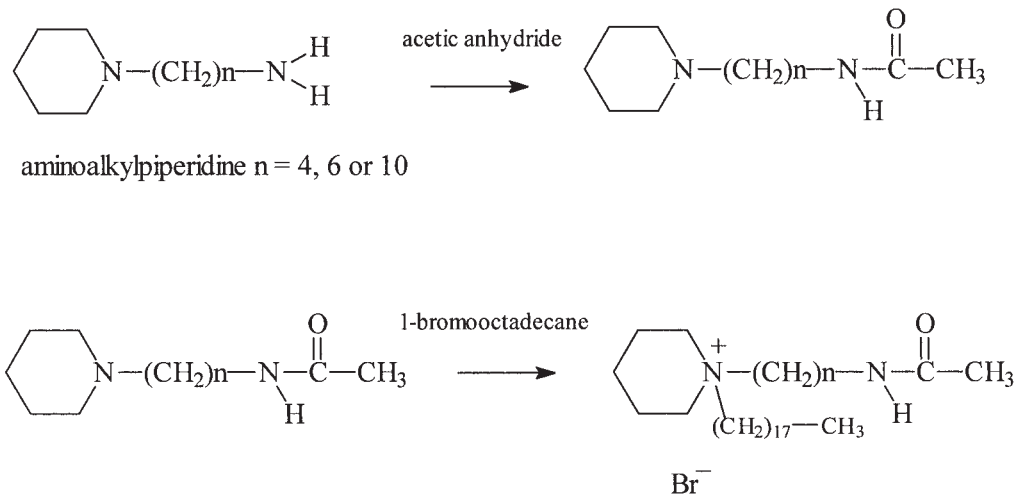


Figure 3 Preparation of the quaternized amide ammonium compounds.

CH_2N^+ and $CH_3(CH_2)_{16}CH_2$ and N^+ ring CH_2]. ^{13}C-NMR ($CHCl_3$, δ, ppm): 13.0 [$CH_3(CH_2)_{17}$], 20.0 ($CH_3C{=}O$), 22 ($NHCH_2CH_2$), 24 [$CH_3(CH_2)_{15}CH_2$], 30 ($NHCH_2CH_2CH_2$), 29.2 [$CH_3(CH_2)_{15}$], 39 ($NHCH_2$), 54 (N + ring CH_2), 59 [$N^+CH_2(CH_2)_{16}CH_3$], 170 ($CH_3C{=}O$).

Microbiological analysis

To investigate the antibacterial efficacy of the surfaces as well as the soluble quaternary ammonium compounds, they were all tested against three organisms: *Escherichia coli* (strain NCTC 9001), a Gram-negative organism; *S. aureus* (strain NCIMB6571), a Gram-positive organism; and *Saccharomyces cerevisiae* (strain NCTC 10716), a yeast. It must be emphasized that only a preliminary microbiological survey was carried out at this stage to show the capability of generating self-disinfecting surfaces, and so any results presented are simply those of a pilot study. The generation of sufficient data to carry out statistically significant experimental findings will form the basis of future investigations.

To evaluate the soluble quaternary ammonium salts against *E. coli* and *S. aureus*, a serial dilution method was employed. Essentially, cultures of each organism were prepared overnight in a nutrient broth at 37°C to give a concentration of 10^8 colony forming units per milliliter (cfu/mL), and they were subsequently diluted to 10^6 and 10^5 cfu/mL. To a $1 \times 10^6 M$ solution of the quaternary ammonium compound (9 mL) was added 1 mL of approximately 10^6 or 10^5 cfu/mL of the appropriate organism, and the resulting mixture was incubated at 25°C for 24 h. Each solution was then serially diluted five times by a factor of 10 each time (1 mL into 9 mL of water), and 0.1-mL aliquots of each dilution were plated out on plate count agar and incubated at 37°C for 24 h. A control experiment was also set up in which no quaternary ammonium compound was included. The colonies were then counted on the plates, with 30–300 colonies per plate, and the colony forming units for the original sample were recorded.

In addition to the two species of bacteria, the soluble quaternary ammonium compounds were tested against the yeast species as previously described. The initial concentration of yeast was a 10^{-1} or 10^{-2} dilution of 10^8 cfu/mL from a 48-h culture grown in a malt extract broth. After incubation, the samples were serially diluted, and 0.1-mL aliquots were plated out on malt extract agar (MEA) and incubated at 25°C for 48 h. The colonies were then counted to give the colony forming units.

Stability test of amide-linked quaternary ammonium compounds with a spread plate method

As a second experiment to test the stability of the amide linkage, the film samples were also laid down on a spread plate culture and incubated at 37°C for 24 h to see if bacterial growth was inhibited. Any zone of inhibition would clearly be attributable to the quaternary ammonium salt being released from the film by hydrolysis of the amide linkage.

Antibacterial activity of amide-bound quaternary ammonium compounds

The immobilized quaternary ammonium salts were investigated for their antibacterial activity against the Gram-positive and Gram-negative organisms, again with a serial dilution method, as described previously. Film samples (2 × 1 cm) were incubated with 1 mL of a bacterial suspension containing 10^5 or 10^6 cfu/mL in 9 mL of H_2O. A piece of ungrafted PE served as a

TABLE I
Antimicrobial Activity of the Soluble Quaternary Ammonium Salt at a Concentration of $1 \times 10^{-6} M$

	S. aureus		*E. coli*	
Sample	Initial cell count = 3.25×10^5 cfu/mL	Initial cell count = 3.25×10^6 cfu/mL	Initial cell count = 6.5×10^5 cfu/mL	Initial cell count = 6.5×10^6 cfu/mL
Control	7.3×10^{3a}	1.3×10^{5a}	3.6×10^{6a}	4.25×10^{6a}
Salt ($n = 10$)	0^a	0^a	0^a	0^a

n = number of methylene groups in the spacer arm.
[a] Viable organisms remaining.

control. Serial dilutions were then prepared as described previously, and 0.1-mL aliquots were plated out and incubated at 37°C for 24 h. The colonies were then counted on a plate containing, with 30–300 colonies per plate, and the colony forming units for the original sample were recorded. The colony forming units gave an estimate of the viable organisms.

Antimicrobial activity of amide-bound quaternary ammonium compounds against *S. cerevisiae*

The immobilized quaternary ammonium compounds were tested against the yeast with a serial dilution method similar to that described previously. Film samples (2 × 1 cm) were incubated with a 1-mL cell suspension (from a 48-h culture diluted to give a final concentration of approximately 10^7 or 10^6 cfu/mL) in 9 mL of water at 25°C for 24 h. The samples were then serially diluted in water as described previously, and 0.1-mL aliquots were plated out on MEA and incubated at 25°C for 48 h. The colonies were then counted to give an estimate of the number of viable cells.

Ability of the self-disinfecting surfaces to withstand successive challenges of bacteria

The ability of a self-disinfecting surface to retain its antibacterial properties after continuous successive challenges of micro-organisms would be an extremely desirable feature of such a material, particularly if it formed part of a surgical prosthesis such as a catheter. In this experiment, therefore, the most effective of the film samples containing a spacer arm of 10 carbon atoms was subjected to a second challenge of bacteria.

The method employed was essentially that described previously, again being based on serial dilution. A piece of untreated PE film again served as a control. After the first 24 h of incubation, the film sample was removed from the solution and washed in 9 mL of water. It was subsequently incubated for 24 h in a fresh solution containing 9 mL of deionized water and a 1-mL cell suspension diluted to a concentration of 10^5 or 10^6 cfu/mL. Both solutions (first and second challenges) were serially diluted, and 0.1-mL aliquots were placed on plate count agar and incubated at 37°C for 24 h. The colonies were counted on the plates, with 30–300 colonies per plate, and the colony forming units of the original sample were recorded. The colony forming units gave an estimate of the numbers of viable organisms.

Effect of the self-disinfecting surfaces on mammalian cells

The Chinese hamster ovary cell line DG44 was routinely grown at 37°C in Dulbecco's modified essential medium containing 10% fetal calf serum, nonessential amino acids (1×), and hypoxanthine/thymidine (1×) together with penicillin/streptomycin.

For the attachment assays, three 1-cm^2 fragments of the films with immobilized quaternary ammonium salts were each placed in a single well of a six-well plate at 37°C. After trypsinization of the cultured DG44 cells, approximately 10^5 cells were introduced into each well. As a control, cells were also grown in wells from which the film was omitted.

RESULTS

Stability of the amide linkage by a dye-binding assay

Under the conditions employed in the assay procedure, no quaternary ammonium salt could be detected in solution, and this showed that the linkage was stable at a neutral pH over a 24-h period.

Spread plate experiment

The results from the experiments in which the pieces of film were placed face down on the spread plate

TABLE II
Antimicrobial Activity of the Soluble Quaternary Ammonium Salt Against *S. cerevisiae*

Sample	Initial cell count = 1.02×10^7 cfu/mL	Initial cell count = 1.02×10^6 cfu/mL
Control	6.75×10^{5a}	3.2×10^{4a}
Salt ($n = 10$)	2.65×10^{3a}	0^a

n = number of methylene groups in the spacer arm.
[a] Viable organisms remaining.

TABLE III
Antimicrobial Activity of PE-Immobilized Quaternary Ammonium Salts with an Initial Cell Concentration of 10^5 cfu/mL

	S. aureus		*E. coli*	
Sample	Initial cell count = 1.48×10^5 cfu/mL	Initial cell count = 1.45×10^5 cfu/mL	Initial cell count = 1.25×10^5 cfu/mL	Initial cell count = 6.5×10^5 cfu/mL
Control	1.5×10^{5a}	1.5×10^{5a}	5.0×10^{2a}	3.0×10^{4a}
PE film, $n = 4$	0^a	0^a	0^a	2.5×10^{4a}
PE film, $n = 6$	0^a	0^a	0^a	1.5×10^{2a}
PE film, $n = 10$	0^a	0^a	0^a	0^a

n = number of methylene groups in the spacer arm.
[a] Viable organisms remaining.

showed no zone of inhibition around the films. This observation indicated that the quaternary ammonium salt was not being released from the polymer over the course of the experiment, confirming the stability of the amide bond. Furthermore, no bacterial growth was detected under either the test sample or control film. The reasons for this are not completely clear, particularly as *E. coli* is a facultative anaerobe.

Antimicrobial effects of a soluble quaternary ammonium salt

Table I shows the results of a soluble quaternary ammonium compound against *S. aureus* and *E. coli*. The results presented in Table I show that at the initial concentrations, the soluble compound was equally effective against both the Gram-positive and Gram-negative organisms, causing complete eradication in both cases. In this experiment, only the amide having a spacer arm length of 10 methylene groups was prepared and tested, as it was thought that the length of the linker arm on a soluble compound should not have any significant effect.

When the same quaternary ammonium compound was tested against the yeast species (Table II), it was highly active when an initial cell concentration of 10^6 cfu/mL was used, again causing complete eradication of the organism. When the initial cell count was increased to 10^7 cfu/mL, a considerable reduction to 2.65×10^3 cfu/mL was obtained.

In the controls shown in Tables I and II, a drop in cell viability was noted. This may well have been due to shock caused by a diluent not yet at room temperature.

Antibacterial effects of the immobilized quaternary ammonium compounds

The results obtained when an initial cell concentration of approximately 10^5 cfu/mL was used for both *S. aureus* and *E. coli* are shown in Table III.

Table IV shows the results of increasing the initial cell concentration to approximately 10^6 cfu/mL.

The results presented in Tables III and IV show that the films with immobilized quaternary ammonium salts did show a cell reduction ability, which may have been either biocidal or biostatic, especially against Gram-positive *S. aureus*. Table III indicates a relationship between the length of the spacer arm and the antimicrobial activity of the quaternary ammonium moiety, complete eradication being observed when the number of methylene groups was 10. However, this factor did not seem to be significant against *S. aureus*, for which the eradication of the organisms was observed in all cases. These findings may suggest that the distance of the quaternary ammonium function from the polymer backbone plays a role in determining the antimicrobial activity of the films. When the initial cell concentration was increased by a factor of 10 (Table IV), a trend could be seen in which increas-

TABLE IV
Antimicrobial Activity of PE-Immobilized Quaternary Ammonium Salts with an Initial Concentration of 10^6 cfu/mL

	S. aureus		*E. coli*	
Sample	Initial cell count = 1.48×10^6 cfu/mL	Initial cell count = 1.45×10^6 cfu/mL	Initial cell count = 1.25×10^6 cfu/mL	Initial cell count = 6.5×10^6 cfu/mL
Control	3.0×10^{6a}	4.5×10^{5a}	1.43×10^{7a}	7.1×10^{5a}
PE film, $n = 4$	1.5×10^{4a}	3.85×10^{5a}	0^a	1.75×10^{5a}
PE film, $n = 6$	4.0×10^{2a}	9.0×10^{2a}	1.2×10^{3a}	5.2×10^{3a}
PE film, $n = 10$	0^a	0^a	8.0×10^{2a}	8.15×10^{3a}

n = number of methylene groups in the spacer arm.
[a] Viable organisms remaining.

TABLE V
Antimicrobial Activity of the PE-Immobilized Quaternary Ammonium Salt Against Successive Challenges of *E. coli*

	E. coli			
Sample	First challenge, initial cell count = 5.05×10^5 cfu/mL	Second challenge, initial cell count = 7.1×10^5 cfu/mL	First challenge, initial cell count = 5.05×10^6 cfu/mL	Second challenge, initial cell count = 7.1×10^6 cfu/mL
Control	3.5×10^{4a}	7.55×10^{5a}	2.5×10^{5a}	5.85×10^{6a}
PE film ($n = 10$)	0^a	1.55×10^{5a}	0^a	1.15×10^{5a}

[a] Viable organisms remaining.

ing the spacer arm did increase the antibacterial activity against the Gram-positive organisms. However, with Gram-negative *E. coli*, no such obvious trend was observed. Table IV shows what appears to be an anomalous result in that the four-carbon spacer unit produced complete eradication of *E. coli* at an initial cell count of 1.2×10^6 cfu/mL. Clearly, further experimentation is required to test the veracity of this result.

When the film containing the 10-carbon spacer arm was subjected to two successive challenges of both species of bacteria, the second challenge did not appear to give any reduction in the numbers of viable *S. aureus*, although some reduction with *E. coli* was obtained. The results of the two successive challenges are shown in Tables V and VI.

The lack of cell reduction obtained with the second challenge of bacteria is a matter for further investigation but might well be explained by the presence of adsorbed organic material on the positively charged membrane surface. Ionically, bound materials such as proteins would effectively mask the quaternary ammonium function.

Activity of immobilized quaternary ammonium functions against the yeast *S. cerevisiae*

Table VII shows the results obtained when the films containing the immobilized quaternary ammonium salts were subjected to a challenge of the yeast species *S. cerevisiae*. In this experiment, the initial inoculum was 10^6 or 10^7cfu/mL, essentially an order of magnitude greater than that used for the bacterial species. The reason for this was the more rapid growth of the yeast culture. For this reason, a direct comparison of the efficacy of the films against this organism and against the bacteria was not possible.

Table VII indicates that when a spacer arm of six or more carbon atoms is present between the quaternary ammonium function and the grafted acrylic acid backbone, the film is effective against a challenge of 10^6 cfu/mL. At a higher concentration of 10^7cfu/mL, however, the films appeared to be far less effective, and this suggests that the size of the inoculum is quite essential for activity to be shown.

Growth of mammalian cells on PE film with immobilized quaternary ammonium salts

The mammalian Chinese hamster ovary DG44 cells neither attached to nor grew on the modified PE film surfaces. In contrast, the cells in the control wells grew normally. This observation may be due to the inhibition of cell attachment caused by the immobilized quaternary ammonium salt, attachment to a surface being essential for cell growth. It is also possible that the cytotoxic properties of the quaternary ammonium functions themselves prevented cellular attachment and growth, and this indicates that it might be toxic toward some mammalian cell lines.

DISCUSSION

A series of self-sterilizing surfaces based on PE were prepared and chemically characterized; a quaternary ammonium function was bound covalently to the polymer backbone by a number of synthetic chemical steps. The quaternary ammonium functions were bound by an amide linkage, which was stable under

TABLE VI
Antimicrobial Activity of the PE-Immobilized Quaternary Ammonium Salt Against Successive Challenges of *S. aureus*

	S. aureus			
Sample	First challenge, initial cell count = 1.75×10^5 cfu/mL	Second challenge, initial cell count = 3.85×10^5 cfu/mL	First challenge, initial cell count = 1.75×10^6 cfu/mL	Second challenge, initial cell count = 3.85×10^6 cfu/mL
Control	1.15×10^{4a}	1.96×10^{4a}	1.85×10^{5a}	1.6×10^{5a}
PE film ($n = 10$)	0^a	1.75×10^{4a}	0^a	1.7×10^{5a}

n = number of methylene groups in the spacer arm.
[a] Viable organisms remaining.

TABLE VII
Activity of the Immobilized Quaternary Ammonium Salts Against *S. cerevisiae*

Film sample	Initial cell count = 1.02×10^7 cfu/mL	Initial cell count = 1.02×10^6 cfu/mL
Control	3.4×10^5[a]	2.2×10^5[a]
$n = 4$	1.67×10^5[a]	1.7×10^4[a]
$n = 6$	8.9×10^4 [a]	0[a]
$n = 10$	1.5×10^4 [a]	0[a]

n = number of carbon atoms in the spacer arm.
[a] Viable organisms remaining.

the experimental conditions employed, and the covalently immobilized salts were active against Gram-positive and Gram-negative organisms, as well as a yeast species, *S. cerevisiae*. The polymers showed antibacterial properties with apparent differences in the number of methylene groups in the spacer arm.

The antibacterial activity of the quaternary ammonium function itself was demonstrated by the antimicrobial action of the soluble quaternary ammonium compounds. In addition, the length of the carbon chain in the spacer arm may well be an important factor; this trend was observed particularly against Gram-positive *S. aureus* (Table IV). At a lower cell concentration (Table III), this factor also appeared as though it may have been important against the Gram-negative species. However, as the cell concentration was increased, no obvious trend could be observed against these latter organisms. The reason for this is not clear at this stage and will require further investigation.

The results from the experiments in which the surfaces were challenged with two successive challenges of bacteria showed that the films were much less effective against the second challenge (Tables V and VI). As mentioned previously, the reason for this may well be due to the surface adsorption of lysed cells or cellular proteins onto the film surface. The positively charged nature of quaternary ammonium compounds makes this a highly likely possibility. This finding may indicate that the use of such a material could be compromised in a real situation in which patients are intermittently exposed to low concentrations of bacteria, such as those with indwelling catheters that may be exposed to body fluids.

The observation that *S. aureus* appeared to be more sensitive than *E. coli* may well have been due to differences in the chemical compositions of the cell walls.

As mentioned previously, on account of the time-consuming nature of preparing sufficient quantities of materials, only a preliminary microbiological pilot study was carried out at this stage, and no attempt was made to produce results of statistical significance. The findings do, however, demonstrate that the chemistry employed is capable of generating materials with antibacterial surfaces. Clearly, the next step in any future investigation will be to determine the antibacterial action of the self-disinfecting surfaces in more detail. As well as providing statistically significant data with the aforementioned organisms, studies will be extended to include other species such as *Pseudomonas aeruginosa* and *S. epidermidis*. A more complete study would also be provided by the inclusion of organisms such as spore-forming bacilli. The investigations do, nevertheless, describe in detail the chemical preparation of an effective self-disinfecting surface and provide sufficient preliminary microbiological data to support further investigation into their potential use in biomedical applications such as catheters, surgical prostheses, surgical materials, containers for sterile liquids, and topical wound dressings.

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