Influence of immobilized quaternary ammonium group surface density on antimicrobial efficacy and cytotoxicity

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ABSTRACT

Bacterial colonization of medical devices causes infections and is a significant problem in healthcare. The use of antibacterial coatings is considered as a potential solution to this problem and has attracted a great deal of attention. Using concentration density gradients of immobilized quaternary ammonium compounds it was demonstrated that a specific threshold of surface concentration is required to induce significant bacterial death. It was determined that this threshold was 4.18% NR\textsubscript{4}+ bonded nitrogen with a surface potential of +120.4 mV. Furthermore, it is shown for the first time that adhesion of constituents of the culture medium to the quaternary ammonium modified surface eliminated any cytotoxicity towards eukaryotic cells such as primary human fibroblasts. The implications of this type of surface fouling on the antimicrobial efficacy of surface coatings are also discussed.

KEYWORDS
Density gradients; quaternary ammonium; antibacterial; cytotoxicity; biological fouling

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Introduction

Increases in population size and average life expectancy along with advances in healthcare have led to a greater demand for medical devices. However, bacterial adhesion and colonization of these devices has become more of a concern as up to 70% of hospital acquired infections (HAI) are associated with medical devices and are complex and costly to treat (Bryers 2008). Combined with the increased prevalence of multi-drug resistant bacteria, infections are a major concern within the medical community (Ioannou et al. 2007; Bryers 2008; Noimark et al. 2009; Vasilev et al. 2009). Widespread antibiotic use has effectively treated countless cases of infection and has had a profound positive impact on public health (Nikaido 2009). However, the overuse of antibiotics, within both the medical and agricultural fields, has been associated with an increased number of multi-drug resistant bacteria (Gould 2009; Nikaido 2009; Noimark et al. 2009). Infections caused by multi-drug resistant bacteria are becoming an increasingly common problem. Although their initial appearance seemed restricted to hospitals, evidence suggests that multi-drug resistant strains of bacteria are prevalent within the general community (de Lencastre et al. 2007). It should also be noted that even bacteria not usually considered to be pathogens, such as \textit{Staphylococcus epidermidis}, can cause nosocomial sepsis (Bryers 2008; McCann et al. 2008).

Short term medical devices, such as catheters, wound dressings and medical ventilators, are frequently replaced in order to remove the devices before bacteria can successfully colonize them. Unfortunately, this adds considerable costs to patient care and can be an inconvenience to patients (Noimark et al. 2009). The situation with permanent implants such as heart valves, hip or knee implants is much more serious since these devices cannot be easily replaced. Infections of such devices can be life threatening and require a substantial medical intervention.

Surface modification techniques have been proposed as a strategy to add intrinsic antimicrobial activity to medical devices to reduce the risk of infection. The addition of quaternary ammonium compounds (QAC) within a coating, in both non-leaching and leaching forms, has been shown to effectively inhibit bacterial growth (Isquith et al. 1972; Tiller et al. 2001; Pidhatika & Rakhmatullina 2015). QAC have been long known for their antibacterial properties (Hegstad et al. 2010). The capacity of QAC to kill bacteria was first noted in 1916 and has been studied and applied to various cleaning applications since (Jacobs et al. 1916). It has been shown that the antimicrobial activity of QAC varies depending on the length and types of the side chains and the conditions of exposure. It is generally accepted that, in solution, long C-chains, combined with positively
charged NR$_4^+$ groups, penetrate cell membranes, and hence interfere with bacterial membrane integrity and ultimately kill the cells (McCubbin et al. 2006; Ioannou et al. 2007; Ferreira et al. 2011). Fabrication of QAC-based surfaces results in a broad-spectrum antimicrobial surface (Alves & Olivia Pereira 2014; Asri et al. 2014). In pioneering work by Tiller et al. (2001), it was concluded that long C-chains on immobilized QAC surfaces was detrimental to the antimicrobial effects of QAC. This is contrary to the effects seen with QAC in solution. Although the exact mechanism of action of immobilized QAC is still under debate (Harding & Reynolds 2014), it has been proposed that the surface charge prompts unfavourable ion exchange with the bacterial membrane (Kügler et al. 2005). If this is true, the minimum inhibitory concentrations (MIC) of immobilized-QAC and MICs in solution would differ (Asri et al. 2014). However, unlike an antifouling surface, surfaces that rely on cationic functionalities for antimicrobial action do not prevent adhesion of proteins on the surface, which in turn can lead to further biofouling (Harding & Reynolds 2014).

This work address two important issues associated with the potential application of immobilized QAC as antibacterial coatings. Firstly, this work aimed to determine the minimal QAC surface immobilization density that is required to inhibit bacterial colonization. To address this question a unique and controlled surface gradient of QAC density was developed. It is hypothesized that there is a threshold of QAC surface density and a minimum positive surface potential is required for a surface to become bactericidal. The second issue tackled in this work relates to the cytotoxicity of QAC-based antibacterial coatings. In the same way that QAC are lethal to bacteria in solution, they can be toxic to eukaryotic cells (Ferk et al. 2007). However, many polycationic antimicrobial agents, including quaternary ammonium based polymers, display minimal cytotoxicity (Milović et al. 2005; Stratton et al. 2009; Peng et al. 2010; Li et al. 2011) when placed on surfaces. Yet, the reason for this apparent biocompatibility has not been directly addressed. Here, the first explanation of why immobilized QAC show limited observable cytotoxicity and how this reflects the antimicrobial efficacy of these surfaces is presented.

**Materials and methods**

**Materials**

Round glass coverslips (13 mm × 0.1 mm in thickness) were used as substrata. Allylamine (98%) and glycidyltrimethylammonium chloride (GTAC; technical grade ≥ 90%) were purchased from Sigma-Aldrich, Castle Hill, New South Wales, Australia. Phosphate buffered saline (PBS), nutrient agar and cold filterable trypicase soy broth (TSB) were purchased from Oxoid, Adelaide, Australia. BacLight® LIVE\DEAD stain was purchased from Invitrogen, Mount Waverley, Victoria, Australia. Complete culture media were prepared using Dulbecco's modified eagle medium (DMEM; purchased from Life Technologies, Mulgrave, Victoria, Australia) amended 10% (v/v) with foetal bovine serum (Atlas Biologicals Fort Collins, CO, USA), 100 IU ml$^{-1}$ of penicillin, 100 mg ml$^{-1}$ of streptomycin, and 0.625 mg ml$^{-1}$ of amphotericin B (Sigma-Aldrich Australia). Resazurin sodium salt was also purchased from Sigma-Aldrich Australia.

**Substratum preparation**

Surface immobilization of GTAC was carried out as depicted in Figure 1a (Cavallaro et al. 2014). Briefly, GTAC was immobilized to plasma polymerized allylamine (ppAA). All substrata were cleaned sequentially with acetone and ethanol, and dried with nitrogen prior to plasma deposition. Allylamine was deposited at a pressure of 2.1 × 10$^{-1}$ mbar and a power of 40 W for 2 min in a plasma reactor described previously (Griesser 1989; Vasilev et al. 2009). GTAC was covalently bound to the plasma deposited amine-rich coatings from a 1 w% GTAC aqueous solution. The solution pH was adjusted to pH 10 using KOH. Samples were vigorously washed in MilliQ water to remove any unattached GTAC and excess KOH and dried using a stream of nitrogen gas.

Concentration gradients of GTAC were created based on controlling the duration of the solution–surface contact (Goreham et al. 2013). A Zaber T-LSR linear motion drive (Zaber Instruments, Vancouver, BC, United States) was utilized to electronically control the dip rate of ppAA modified substratum into a GTAC solution as prepared above. Samples were dipped at a continuous speed of 1 mm h$^{-1}$ along the z-axis (Figure 1b).

**Surface analysis**

X-ray photoelectron spectroscopy (XPS) was used to quantify both the presence of the QAC on the surface and the effect of sterilization of the surface. A SPECS Sage XPS (SPECS Surface Nano Analysis, Berlin, Germany) equipped with a 200 kW monochromatic Mg source was used for acquisition. High resolution spectra were recoded for pertinent photoelectron peaks at a pass energy of 20 eV. Data were analysed using CasaXPS V2.3.14 (Casa Software Ltd., Teignmouth, UK) and all binding energies were charge corrected relative to C-C at 285.0 eV. A N1s spectrum was used to analyse the change in the NR$_4^+$ peak along the concentration gradient.
A colloid probe AFM technique was used to determine the surface potentials at different points along the gradients. A ‘NT-MDT NTEGRA SPM’ AFM was used for all measurements with samples submerged in 1 × 10^{-2} M KCl at pH 7. The deflection upon approach with a curved hydrophilic Si AFM tip was measured and the surface potential calculated (Mierczynska et al. 2012).

**Bacteria**

*E. coli* (ATCC 25922) and *S. epidermidis* (ATCC 35984) were used as model organisms. The bacteria were grown from single colonies in broth cultures, incubated at 37°C overnight. An inoculum of 400 μl of 1 × 10^6 CFU ml⁻¹ of bacteria, based on the McFarland standard, was incubated on the treated substrata for 4 h. Samples were washed with PBS to remove any non-adhered bacteria. Adhered bacteria were stained using LIVE/DEAD stain and quantified by counting the number of live (green) and dead (red) bacteria per field of view. Fluorophores were excited using a 470 nm CoolLED light source (CoolLED Ltd, Andover, UK). A Nikon B-2A fluorescence filter cube set (Nikon Instruments, New York, USA) was used for imaging. The influence of environmental factors on bacterial–surface interactions was assessed by testing homogenous QAC surfaces with samples in low nutrient (PBS) and high nutrient environments (TSB). Experiments were conducted ensuring that all surfaces remained completely submerged in liquid throughout the washing and staining procedures so as to not allow the bacteria to dry out.

**Figure 1.** (a) Schematic representation of (i) plasma deposition of allylamine for generation of (ii) amine-rich ppAA coated surfaces; (iii) immobilisation of GTAC to primary amine groups for generation of homogenous ppAA+GTAC surfaces. (b.i) Amine-rich surfaces were dip coated into (ii) GTAC solution to generate (iii) QAC concentration gradients.
Differences between the numbers of dead bacteria exposed to the concentration gradient compared to the ppAA end (1.5 mm) were analysed using a one-way ANOVA with a Dunnett’s post hoc test on GraphPad Prism 5 for Windows (GraphPad Prism 5, Graphpad Software Inc., San Diego, CA, USA).

Mammalian cells

Primary human dermal fibroblast cells were cultured in the complete culture medium prepared as above. To each well of a 24-well plate containing coated 13 mm glass coverslips, 20,000 cells were added and incubated for 72 h. A resazurin reduction assay was used to measure relative metabolic activity (O’Brien et al. 2000). Resazurin was prepared in PBS (110 mg ml\(^{-1}\)) and diluted 1:10 in complete culture medium. To each sample 500 μl were added, and plates were incubated for 4 h; then 200 μl of each well were transferred into a 96-well plate and fluorescence at 544/590 nm measured using a FLUOstar Optima plate reader (BMG LABTECH Pty. Ltd Victoria, Australia). All statistical analysis was performed on GraphPad Prism 5 for Windows using an unpaired t-test to compare ppAA+GTAC to the ppAA control.

Effect of fouling

The capacity of constituents of the growth medium to adsorb to the GTAC modified surfaces was investigated using a quartz crystal microbalance with dissipation monitoring (QCM-D). A Q-sense E4 QCM-D instrument was used in combination with 14 mm diameter quartz sensors with gold electrodes on either side of the sensor. All sensors were pre-washed with Milli-Q filtered water at a constant flow rate of 0.1 ml min\(^{-1}\) until stable. TSB was flowed through the QCM chamber until both the vibrational dissipation and frequency of the sensors stabilized. Changes in the vibrational frequency (Δf), post washing with Milli-Q filtered water are indicative of changes in sample weight due to adsorption of molecules form the solution.

Results and discussion

Immobilization of QAC

QAC modified surfaces were generated via the addition of a 1 wt% solution of GTAC to ppAA coated substrata (Cavallaro et al. 2014). The binding of GTAC was confirmed via high resolution XPS scans in the bonding energy range of the N1s region. Quantification of the surface concentration of QAC was determined by deconvolution of the N1s spectra. Figure 2a shows high resolution XPS N1s spectra of ppAA and ppAA+GTAC. The ppAA N1s spectrum (Figure 2a.i) can be fitted with a single component at 399.8 eV which is typically done with plasma polymer films due to the very small differences in binding energies of N-C bonded nitrogen species (Vasilev et al. 2010; Michelmore et al. 2011) which makes fitting uncertain. After addition of GTAC (Figure 2a.ii) a new peak at 402.4 eV appears, which indicates the presence of the quaternary ammonium cation (Kistamah et al. 2009). The atomic percentage of quaternary ammonium groups on these uniformly modified surfaces was 6.89% of the total nitrogen.

Antimicrobial activity

To determine the antimicrobial efficacy of the uniformly modified ppAA+GTACs towards bacteria, cultures of E. coli and S. epidermidis were incubated on the surface for 4 h. E. coli and S. epidermidis were chosen as test organisms due to their medical relevance and different cellular envelope structures, S. epidermidis being Gram-positive and E. coli Gram-negative (OGara & Humphreys 2001; Russo & Johnson 2003).

The LIVE\(\)DEAD stain assay was used determine the viability of the individual bacterial cells adhered to the surface. All bacteria were stained with Syto9 (green), whereas propidium iodine (red) only enters dead cells with permeable membranes (Joux & Lebaron 2000). Figure 2 shows the effect of the immobilized QAC on adhering bacterial viability. Quantification of the number of green-only stained cells showed that 96.6% of the E. coli cells adhered to the untreated glass (Figure 2c) and 97.9% of the E. coli cells adhered to the ppAA surfaces (Figure 2e) were viable. A reduction of 70.7% in the number of viable E. coli cells adhered to the ppAA+GTAC surface was observed as shown by the large number of propidium iodine (red) stained cells (Figure 2g). Figure 2b, d and f shows the interaction of S. epidermidis with untreated glass, ppAA and ppAA+GTAC modified surfaces, respectively. Interestingly, the ppAA+GTAC coated substrata had no effect on the bacteria since S. epidermidis appeared viable regardless of the surface type.

The ability of the GTAC modified surface to induce membrane permeability in E. coli and not in S. epidermidis was attributed to differences in the respective bacterial cell envelope (Willey 2008; Silhavy et al. 2010). Lipopolysaccharide (LPS) is the major component of the outer cell membrane of Gram-negative bacteria, such as E. coli. The positively charged groups of ppAA+GTAC can interact with the outer membrane in two ways. First, the charged groups may repel ions, such as Mg\(^{2+}\), used for charge stabilization (Tiller et al. 2001; Groisman et al. 2013). Removal of such ions from the cell membrane
This interaction could alter the structure of, or potentially remove, the LPS, which can disrupt membrane integrity and lead to cell death. Here it should be noted that killing of Gram-negative bacteria leads to the release of the LPS will result in its destabilization and lead to an increase in cell membrane permeability. Secondly, the charge may enhance the adhesion forces between the bacteria and the surfaces (Asri et al. 2014) by attracting LPS molecules.

Figure 2. (a) XPS N1s high resolution spectra of (i) ppAA and (ii) ppAA+GTAC. Fluorescent images after LIVE/DEAD staining of bacteria incubated with the corresponding surfaces showing S. epidermidis grown on (b) glass, (d) ppAA and (f) ppAA+GTAC and E. coli grown on (c) glass, (e) ppAA and (g) ppAA+GTAC.
which results in an increased immunological response on the part of the host (Vanaja et al. 2015).

Although Gram-positive and Gram-negative bacteria tend to have net negative charges, Gram-positive bacteria, such as *S. epidermidis*, have thicker cell membranes consisting of high levels of peptidoglycan (van der Wal et al. 1997; Silhavy et al. 2010). These high levels of peptidoglycan give the bacterial cell membranes greater rigidity and thickness. This could be one of the reasons why *S. epidermidis* might be less susceptible to membrane destabilization caused by the surface grafted QAC (Kenawy et al. 1998; Gottenbos et al. 2001). Due to the lack susceptibility of the *S. epidermidis* to the immobilized QAC, only *E. coli* was used for further investigation.

**Minimum bactericidal concentrations**

Next, the role of QAC surface immobilization density on bacterial viability was examined. These gradients allowed the use a single substratum to screen the entire achievable concentration range of surface bound GTAC. To create such gradients, the time dependent nature of the epoxide-amine reaction was exploited. A precise dip-coating technique was used to control the times of contact between the amine modified surface and the solution of GTAC. The method is schematically depicted in Figure 1b and was used previously by the authors to create nanoparticle density gradients (Goreham et al. 2013). For the studies reported here, a 13 mm round coverslip was immersed at a rate of 1 mm min$^{-1}$. This rate of immersion allowed for a maximum concentration of quaternary ammonium functionalities on the side of the substratum that remained in the solution for longest time (13 h) while the opposite side of the substratum had no GTAC at all.

Figure 3a shows the surface concentration of NR$_4^+$ groups along the gradient determined deconvolution of the N1s high resolution XPS spectra. The 13 mm position remained in the solution of GTAC for the longest time (13 h). At this position the NR$_4^+$ group concentration was 6.17% of the total nitrogen functionalities. By reducing the time of immersion towards position 0 mm, the NR$_4^+$ group density gradually decreased. At the 1.5 mm position, only 1% of the total nitrogen was in the form of quaternary ammonium ion. The presence of small amounts of quaternary ammonium functionality on the bare plasma polymer film is not surprising since the process is known to deliver complex surface chemistries different from those produced by conventional polymerization techniques (Vasilev et al. 2010).

The surface potential along the gradient was evaluated by AFM based surface force measurements and is presented in Figure 3b. The surface charge was calculated from the approach curves using the DLVO model. The surface potential on the ppAA side of the surfaces (the 1 mm position) was in the range of 70 mV which is consistent with previously published studies (Mierczynska et al. 2012). By increasing the GTAC surface grafting density, the positive surface charge gradually increased to above 150 mV at the highest NR$_4^+$ group concentration.
This increase in the positive surface charge was expected since the NR$_4^+$ has greater protonation than the amine group (Kistamah et al. 2009). As could be anticipated, the increase in the surface potential followed the same trend as the density increase in NR$_4^+$ group surface density determined by XPS (Figure 3a).

*E. coli* was incubated with the QAC surface concentration density gradient following the same procedure as described for uniformly coated surfaces. Figure 4 shows images of LIVE\DEAD stained *E. coli* cells attached to the surface from six evenly spaced points along the gradient, corresponding to positions 1.5, 3.5, 5.5, 7.5, 9.5 and 11.5 mm. The first observation is that there is no statistically significant difference in the total number of cells that adhered to each position along the gradient (Supplementary material, Figure S1). This is to be expected since the bacteria were cultured under low nutrient and slow growth conditions. However, the viability of the attached bacteria was different along the gradient. At low NR$_4^+$ group concentration, e.g., positions 1.5 and 3.5 mm, mostly viable, Syto9 (green) stained cells are observed. When the NR$_4^+$ group surface density increased (position 5.5 mm and above) a high proportion of propidium iodine stained (red) dead cells can be seen (Figures 4 and S2).

Quantification of the percentage of dead (red stained) cells was obtained via ImageJ software (ImageJ, National Institute of Health, Bethesda, MD, USA) analysis and is plotted in Figure 3a. Consistent with the images in Figure 4, the surface had no antibacterial properties on the bare ppAA end of the gradient (the 1.5 mm position) and low NR$_4^+$ group concentration (the 3.5 mm position). As the concentration of NR$_4^+$ groups along the gradient increased, the percentage of dead bacteria also increased. Dead bacteria (~20%) started appearing on the surface at the 5.5 mm position. At the 7.5 mm position and above nearly 70% of the attached bacteria were non-viable. Interestingly, there was no significant difference between positions at 7.5, 9.5 and 11.5 mm when compared to each other (*p* > 0.05). This indicates that there may be a threshold of surface concentration density of quaternary ammonium groups that is required to kill bacteria. This threshold in the case of surface immobilized GTAC is 4.18% (of total nitrogen) with a surface charge of +120.4 mV. This result correlates with published studies which claim that a specific surface
lack of effect of the QAC on metabolic rates hints towards differences in culture conditions influencing the surface and cell interactions. Bacterial culture studies were carried out in nutrient deficient conditions (i.e., PBS) while HDF were grown in a complete cell culture medium. It was possible that proteins and other nutrients from the cell culture medium adsorb to the surface almost immediately after contact and in this way mask the immobilized GTAC molecules responsible for bacterial cell death. This hypothetical scenario is depicted in Figure 6a.

To test this hypothesis, in situ QCM-D measurements which are presented in Figure 6b were made. Sensors coated with ppAA and ppAA+GTAC were incubated in TSB. TSB is a bacterial culture medium containing various nutrients such as proteins and sugars. As seen from the seismogram, almost immediately after introduction of TSB, a significant amount of the medium adsorbed to the sensor surface resulting in a negative frequency change. Some of this material was so strongly attached that it did not desorb after an exhaustive rinse with water. This material which was strongly attached to the ppAA+GTAC modified sensor is estimated at 21.4 ± 1.1 ng and is sufficient to form a thin masking layer on top of the quaternary ammonium groups.

Surface fouling

To evaluate how the adsorbed constituents of the culture medium affect the capacity of surface immobilized QAC to kill bacteria, *E. coli* was grown in nutrient-rich culture broth (TSB). Figure 6c shows images of *E. coli* cells grown on untreated glass, ppAA and ppAA+GTAC for 4 h at 37°C and stained with the LIVE/DEAD assay. Surface immobilized QAC incubated with nutrient-rich medium did not decrease *E. coli* growth and viability. This can be attributed to two effects and the interplay between them. First, the adsorption to the surface of nutrients from the medium forms a thin layer on top of the immobilized GTAC molecules which masks any antibacterial properties of the NR₄⁺ groups. Second, the continuous proliferation of the bacteria in nutrient-rich medium not only leads to production of extracellular matrix components which further masks the QAC groups, but also allows for repair of any damaged cell surfaces (Lund et al. 2000; Virto et al. 2005).

This study makes important contributions to elucidating the use of surface grafted QAC for fabrication of antibacterial surfaces. Using surface density gradients of QAC, engineered at the molecular level, it was demonstrated that a specific threshold of GTAC density is required to achieve antibacterial properties. This specific threshold relates to a NR₄⁺ group concentration of 4.18% of total nitrogen and a surface charge density must be achieved to induce significant antimicrobial effects (Kügler et al. 2005).

Cytotoxicity

The biocompatibility of biomedical devices is one of the most important factors that determine the usefulness of a certain device (Abdull Rasad et al. 2010). To test the cytotoxic potential of the GTAC modified surfaces, primary human dermal fibroblasts (HDF) were used. It should be noted that these cells are primary cells and not immortalized cell lines. Fibroblasts are key in wound healing and are used as an established model for assessing device biocompatibility and potential cytotoxicity (Yang et al. 2002; Werner et al. 2007; Bose & Lau 2009; Wang et al. 2013; Taheri et al. 2014). Figure 5 shows the metabolic activity (assessed using the resazurin assay) of HDF cultured on ppAA+GTAC at the highest surface density and on controls of ppAA, glass slides and tissue culture plates (TCP). There was no statistically significant difference between cells grown on ppAA and ppAA+GTAC (p > 0.05). The metabolic activity of the cells cultured on ppAA+GTAC was also very similar to those on TCP. This result and the ability of the cells to reach full confluence with no observable cellular debris (Figure S3) indicates that the GTAC modified surface, even at the highest surface concentration, did not have any adverse effect on HDF.

The fact that the GTAC modified surfaces were lethal to bacteria but were not toxic to HDF was initially surprising. Indeed, the differences in the cell membrane structures may play a role in the interaction between the cells and the surfaces. (Anderson et al. 2012) However, the complete

Figure 5. Relative metabolic activities (mean ± SEM) of human dermal fibroblasts grown on ppAA and ppAA+GTAC as a percentage of the activity on tissue culture plates showing no statistical difference between ppAA and ppAA+GTAC (p > 0.05). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

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The effect of growth medium on the efficacy of QAC-based coatings is not widely reported in the literature but elucidation of this phenomenon is important for the understanding of when and how such antibacterial coatings can be used. Using QCM-D measurements, it was revealed that the loss of surface activity is due to the adsorption of constituents from the growth medium. Studies were conducted under low nutrient conditions similar to those used repeatedly throughout the literature for the antibacterial assessment of QAC-based coatings (Gottenbos et al. 2002; Lee et al. 2004; Milović et al. 2005; Li et al. 2006, 2011). However, when a rich growth medium was used (TSB), the surface completely lost its antibacterial properties.

The effect of growth medium on the efficacy of QAC-based coatings is not widely reported in the literature but elucidation of this phenomenon is important for the understanding of when and how such antibacterial coatings can be used. Using QCM-D measurements, it was revealed that the loss of surface activity is due to the adsorption of constituents from the growth medium.
The authors believe that the same phenomenon will be observed with other types of antibacterial coatings based on grafted compounds, except when the compounds have intrinsic low fouling properties. These findings do not necessarily render the QAC based coatings and other antibacterial surfaces achieved through surface grafting bactericidal compounds ineffective in medical device applications, as these coatings are still capable of protecting the device surface before the device is implanted into the body. This could be beneficial since it will ensure that viable bacteria are not able to be introduced with the device itself. Once placed into the body, the surface of the device will foul from the constituents of the body fluids and lose its antibacterial properties. However, any potential cytotoxicity to body cells and tissue will also be lost, which would render the device safe.

Conclusions

Concentration gradients of immobilized QAC were used to assess the critical surface concentration of NR₃⁺ groups required to kill attaching Gram-negative pathogens. It was demonstrated that there is a specific threshold of NR₃⁺ group concentration of 4.18% and surface charge of +120.4 mV required to induce death in the case of E. coli. It was also demonstrated that any surface-associated antibacterial properties are lost in nutrient-rich environments. This loss of activity is caused by the adsorption of constituents to the surface which form a thin layer on top of the quaternary ammonium groups. It is speculated that antibacterial coatings based on other types of grafted compounds may have the same fate, thus the implications of the findings reported here may be much broader. In terms of applications for medical devices, these coatings would retain their antimicrobial efficacy until they come into contact with the complex fluids within the body. The latter might be beneficial as this rapid fouling would mask any potential toxic effects of the surface after entering the body, decreasing the risk of any bio-incompatibility. Herein the biocompatibility of the coatings in cultures of HDF were non-cytotoxic. However, any effects on immunological responses or other secondary effects, such as the release of LPS upon killing Gram-negative bacteria, need to be assessed before using ppAA+GTAC on implantable medical devices.

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