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THE BINDING OF URINARY COMPONENTS AND UROPATHOGENS TO A SILICONE LATEX URETHRAL CATHETER

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Abstract

The adhesion of uropathogens to urethral catheters initiates the infectious process which causes morbidity in a large patient population. Catheters were examined by X-ray photoelectron spectroscopy after use in three patients and following incubation in vitro with human urine, urea and creatinine, and found to have adsorbed a conditioning film containing carbon, nitrogen and in some cases phosphorous, calcium and sodium containing components. The binding of three strains of uropathogenic bacteria to the catheter surface was significantly influenced by the suspending fluid composition.

Key Words: Uropathogens, adhesion, silicone latex urethral, catheter, urinary, components.

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Introduction

The ever expanding reliance on biomaterials in health care makes an understanding of their properties of great importance to clinicians and scientists. Urethral catheters are one of the most commonly used prosthetic devices, and their employment is associated with a high number of infections, especially nosocomial (Givens and Wenzel, 1980). Whilst it is recognized that bacterial adhesion and biofilm formation forms an important stage in the infectious process, many unanswered questions remain regarding the early interaction between the organisms, host components and biomaterial device.

It has been shown that a conditioning film forms on the surface of an implanted prosthesis, for example a hip joint, when it comes into contact with tissue components (Gristina, 1987). In the urinary tract, the influence of proteins and urine on bacterial adhesion to polymers has been demonstrated previously (Hawthorn and Reid, 1990), but the adsorption of a urinary conditioning film onto the catheter surface has never been examined using analytical techniques. Whilst microscopy can provide useful information demonstrating bacterial biofilms and encrustations (Cox et al. 1989; Reid et al. 1989), analytical chemistry techniques are required to examine chemical groups adsorbed to the biomaterial surface.

The purpose of the following study was to utilize X-ray photoelectron spectroscopy (XPS) to examine the chemical elements on a silicone coated latex urinary catheter, before use, after implantation in humans and after in vitro incubation with urinary components. In addition, the influence of urinary components on adhesion of uropathogens was tested, using three isolates representative of species which cause most infections in catheterized patients.

Materials and Methods

Catheters.

A latex urethral catheter coated with silicone and containing fluorine components (TFX Medical, Canada) was used throughout this study. Catheters were examined after being implanted for 1, 3 and 7 days into adult patients, hospitalized in St. Joseph's Health Centre, London, Canada. The patients did not have a urinary tract infection, were not receiving antibiotics during this period, and they were discharged from hospital at the time of catheter removal.

XPS Studies.

XPS is a valuable tool for examining the chemical composition of a surface (Rouxhet and Genet, 1991). In brief, irradiation by an X-ray beam induces ejection of electrons from a surface. The kinetic energy of the emitted electrons is analyzed and the binding energy is determined. A spectrum of peaks is produced (Gelius et al. 1970; Clark and Dilks, 1978, 1979; Dilks, 1981; Briggs, 1990), each being representative of an element (eg. carbon, nitrogen). High resolution analysis of these peaks can give an indication of the chemical functional groups present on the surface. In addition, the areas of the most important XPS-peaks were quantitated after linear back ground subtraction and correction for sensitivity factors and subsequently used to obtain the elemental composition of the surfaces.

In this study, XPS was performed on a control catheter surface, three devices used in patients, and those used for *in vitro* experiments (described below). Analysis of samples was carried out using the SSX-100 Surface Science Laboratory X-ray photoelectron spectrometer which uses monochromatized Al Kα X-rays capable of being focused to a spot size of 150 μm (Foerch et al. 1990). The suspension of a metal grid above the sample combined with a variable low energy flood gun was found to provide the best control over sample charging effects, allowing high resolution XPS spectra to be obtained. For the elemental XPS analysis, a spot size of 600 μm and a pass energy of 150 eV were used. These were reduced to 150 μm and 48 eV respectively for the high resolution C_{1s} spectra. Each test was carried out in triplicate on the same sections of catheter (1 cm from tip) and the results were presented as mean and standard deviation.

Bacterial adhesion assay.

Three strains of bacteria were used: catheter isolates *Proteus mirabilis* and *Staphylococcus epidermidis* 1938, and urinary strain *Escherichia coli* Hu734. These were stored at -70°C and cultured in brain heart infusion yeast extract broth overnight prior to use. Concentrations of approximately $1x10^8$ organisms per ml suspending fluids were incubated with 1cm sections of catheter for 24 hours at 100 rpm in a 37°C rotary shaking water bath.

The suspending fluids comprised filter sterilized human urine collected (pH 5.5) from a healthy adult female first morning and in the afternoon. Urea (Difco, Detroit) was made up in saline (pH 7.1) to concentrations of 200 and 800 mM and creatinine (BDH Ltd, Poole, England) was made up to 4 and 20mM. The concentration ranges for urea and creatinine were those found in normal adult urine (Tietz, 1990). After incubation, the catheter sections were subjected to washing in phosphate buffered saline and the adherent bacteria were removed for viable plate counting by sonications in an ultrasonic waterbath for 3 minutes. The number of viable bacteria per section was recorded. Each experiment was carried out in triplicate with data being presented as mean and standard deviation.

Electron microscopy.

Randomly selected specimens representative of the adhesion patterns observed were fixed in 5% glutaraldehyde, dehydrated in alcohol and sputter coated in gold, and examined under a ISI DS-130 scanning electron microscope (Japan), as per Reid et al. (1989).

Results

Figure 1 represents the C_{1s} spectra for the control catheter and those which had been implanted into patients. The C_{1s} spectrum for the control sample showed evidence of at least three different types of carbon atoms. The peak at the lower binding energy (285.0 eV) represented the hydrocarbon structure of the polymer. The second lower intensity peak (286.6 eV) showed the presence of oxygen functional groups. The third, higher binding energy peak (292.3 eV) was typical of CF_2 bonds and characteristic of the fluorine containing polymer used. Exposure to the patient and subsequent XPS analysis showed changes in the chemical composition of the catheter surface; several fold increase in nitrogen and appearance of calcium and phosporous (Table 1). Statistical

analyses by one way ANOVA showed there to be a significant difference in the N/C ratios ($P<0.03$) and a multiple comparison's Fisher's least significant difference test showed the control to significantly differ from the 1 day specimen and to differ marginally from the 3 and 7 days specimens. After one day, the tail of the C_{1s} peak was seen to broaden and included at least one other Gaussian component. This was accompanied by a small decrease in the O/C ratio and suggested a loss of oxygenated surface species or possibly a rearrangement of chemical groups, or other changes due to deposition of a conditioning film. The binding energy separation between peaks suggested a conversion from C-O (perhaps alcohol or ether groups) to more acidic O=C-O groups located around 288.1 eV.

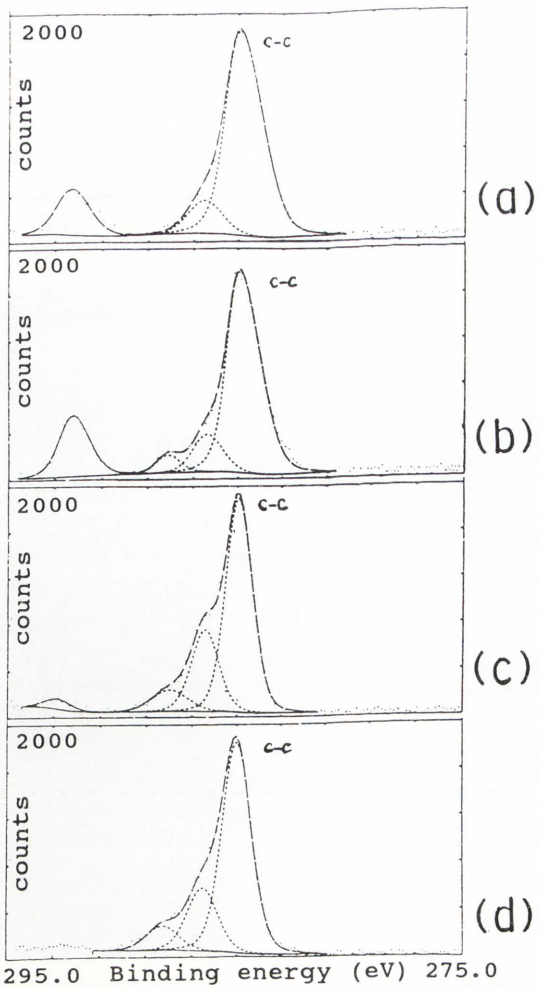

Figure 1. This represents the C_{1s} spectra for (reading from top to bottom, a to d) the control catheter and those implanted into patients for 1 day (b), 3 days (c) and 7 days (d).

After a three day exposure to the patient, XPS indicated further changes (Fig 1c). The intensity of the high binding energy component at 292.3 eV (C-F groups) decreased and was accompanied by an increase in the C-O components (286.6 eV).

After 7 days, the C_{1s} spectrum indicated a reduction in fluorinated groups from the outer surface (F/C ratios, $P=0.02$); alternatively, it could be envisaged that the original catheter surface had been completely covered and/or hidden under a conditioning film. This surface layer appeared to be relatively thick (>10nm). The increase in nitrogen concentration is apparent in the C_{1s} spectra as an intensity increase at a binding energy separation of 1 eV from the main hydrocarbon peak. The detection of silicone on the surface (Si/C ratio) was seen to decrease with longer exposure to the patient ($P<0.05$), and this is another indication of the coverage of the catheter surface with a conditioning film.

Incubation <u>in vitro</u> with am and pm urine ($P<0.05$), urea and creatinine ($P<0.05$) led to increased nitrogen adsorption (table 1). Larger amounts of chlorine ($P=0.01$), sodium ($P=0.5$),

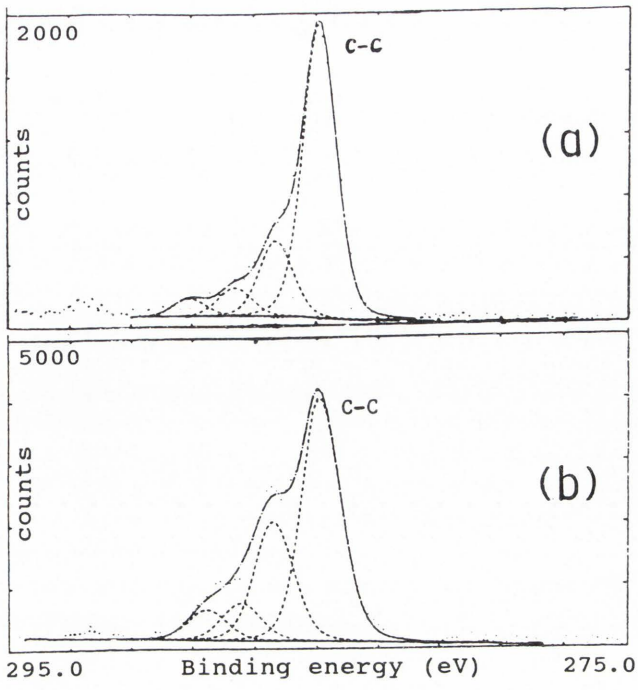

Figure 2. This represents the C_{1s} spectra for the catheter incubated in 200mM urea (a: top) and 20mM creatinine (b: bottom) for 24 hours.

calcium (P=0.02) and phosphorous (P=0.02) were found on the surface incubated with afternoon urine compared to morning urine. After 24 hours exposure to the 200mM urea concentration, the catheter surface was seen by XPS to have a C_{1s} peak (figure 2a) with a tail indicative of increased carbon-oxygen bonding (286.6 eV), and a highly reduced presence of fluorinated groups (292.3 eV). The catheter surface exposed to creatinine showed a quite different XPS spectrum (Figure 2b), with a decreased intensity hydrocarbon peak (285.0 eV), a highly increased carbon-oxygen bonding (286.6 eV), as well as the reduced presence of a carbon-fluorine peak.

The bacterial adhesion experiments, presented in Table 2, demonstrated high levels of binding, especially for the strain of P. mirabilis (Figure 3)($P<0.05$). Two way ANOVA showed there to be highly significant differences in the adhesion patterns amongst the three strains ($P=0.0001$), with adhesion in PBS being significantly greater than those in the other fluids ($P=0.0001$) except for Proteus in 20mM creatinine. Unlike E. coli and S. epidermidis whose adhesion was decreased in higher concentrations of urea and creatinine, the Proteus strain adhered more than two fold greater with increased concentrations of these compounds. There was no difference in the adhesion of the three strains to am and pm urine.

Table 1. Elemental compositions by XPS of silicone latex urinary catheters before and after implantation into a patient up to 7 days and exposure to urea and creatinine for 24 hours.

	O/C	N/C	Cl/C	Si/C	Na/C	Ca/C	P/C	F/C
Control	0.37 (0.04)	0.017 (0.018)	0.009 (0)	0.14 (0.002)	0.02 (0.001)	-	-	0.26 (0.09)
1 day	0.29 (0)	0.07 (0.003)	0.008 (0.001)	0.11 (0.01)	0.02 (0.002)	0.002 (0.002)	0.01 (0)	0.22 (0.14)
3 days	0.37 (0)	0.04 (0)	0.010 (0.002)	0.15 (0.02)	0.01 (0.001)	0.005 (0)	-	-
7 days	0.21 (0.06)	0.04 (0.03)	0.018 (0)	0.07 (0.01)	0.03 (0)	-	0.012 (0)	-
Urine am	0.37 (0)	0.08 (0.001)	0.005 (0)	0.12 (0.01)	0.009 (0)	0.004 (0.001)	-	0.19 (0.1)
Urine pm	0.49 (0.05)	0.09 (0.06)	0.024 (0)	0.05 (0.01)	0.04 (0)	0.11 (0.01)	0.07 (0.03)	0.18 (0.05)
Urea 200mM	0.38 (0.03)	0.03 (0.001)	0.01 (0.004)	0.08 (0.01)	-	0.02 (0)	0.03 (0)	0.14 (0.03)
Urea 800mM	0.34 (0.04)	0.04 (0.014)	0.02 (0)	0.09 (0.01)	-	0.03 (0)	0.02 (0)	0.16 (0.06)
Creat 4mM	0.43 (0)	0.08 (0)	0.04 (0)	0.10 (0.001)	0.07 (0)	0.02 (0)	0.009 (0.001)	0.11 (0.02)
Creat 20mM	0.40 (0.015)	0.07 (0.015)	0.02 (0)	0.08 (0.001)	0.03 (0)	0.02 (0)	-	0.17 (0.13)

(standard deviation based upon 3 specimens)

- = none detected, thus no ratio given

Table 2. Uropathogenic adhesion to urinary catheter after 24 hours incubation at 37°C.

	No. Adherent Viable Bacteria per cm catheter		
Conditions	E.coli	P.mirabilis	S.epidermidis
PBS	$1.3x10^{6}\pm0.2$	$1.1x10^{8}\pm0.09$	$7.0x10^{4}\pm0.5$
Urine am	$1.9x10^{5}\pm2.3$	$1.4x10^{7}\pm0.4$	$7.0x10^{4}\pm1.1$
Urine pm	$3.0x10^{5}\pm1.0$	$2.3x10^{7}\pm0.3$	$2.1x10^{4}\pm1.1$
Urea 200mM	$3.8x10^{5}\pm0.6$	$6.1x10^{6}\pm2.8$	$6.0x10^{1}\pm3.2$
Urea 800mM	$1.1x10^{2}\pm0.3$	$1.5x10^{7}\pm0.7$	$1.2x10^{1}\pm0.6$
Creatinine 4mM	$2.1x10^{5}\pm0.7$	$5.3x10^{6}\pm2.7$	$3.8x10^{3}\pm0.6$
Creatinine 20mM	$1.6x10^{5}\pm0.3$	$1.3x10^{8}\pm0.3$	$3.2x10^{3}\pm1.2$

Mean values from triplicate experiments ± standard deviations

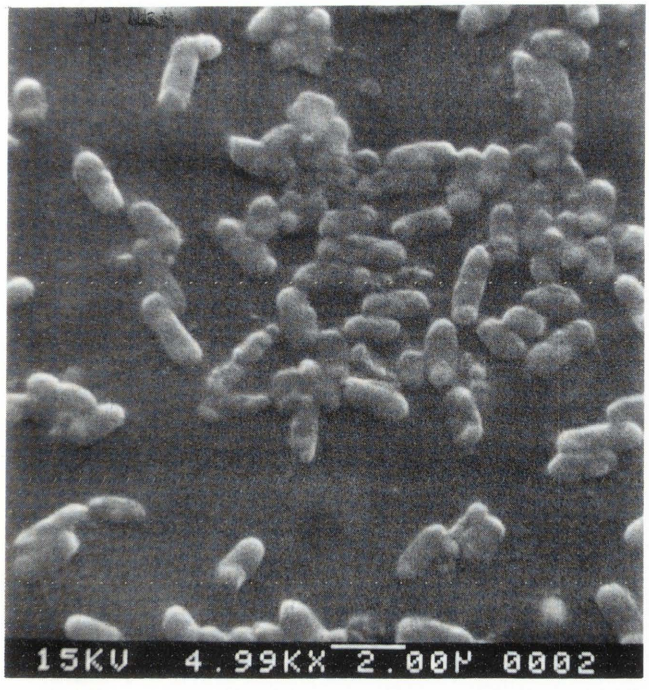

Figure 3. This scanning electron micrograph illustrates the high degree of adhesion of Proteus mirabilis to the surface of the prosthesis after 24 hours incubation. Bar = 2.0 μm.

Discussion

The latest findings demonstrated the presence of a conditioning film on the surface of three urinary catheters collected from patients over a seven day period, and on the surface of catheters incubated in vitro with urinary components for 24 hours. The patient specimens and those incubated with urine, urea and creatinine showed a film with increased carbon and nitrogen containing groups, along with small quantities of calcium and phosphorous. Whilst the precise molecular nature of the film has not been determined, the data does indicate that the catheter surface is quite altered compared to its original chemical structure. Practisioners in the field might not be surprised by this finding as catheters removed from patients often "feel and look" different. However, these findings, albeit only with three patient specimens, are to our knowledge, the first such documentation of actual elemental changes on urinary catheters. The impression that a urinary catheter reacts to approaching bacteria in one way or another depending upon it's surface hydrophobicity or composition as a manufactured product (Roberts et al. 1990) is misguided, as upon insertion into the body, this surface alters rapidly.

The question could be asked are the changes due to a urinary film or

substances leaching from the polymers? Whilst this study cannot fully verify that the film is urinary in origin, there is further supportive data from an analysis of urine air dried onto glass slides and processed by XPS (data not published in full). This showed a carbon peak at 286.6 eV, over twofold more carbon-nitrogen (ratio 0.2 versus 0.086), and the presence of carbon-calcium (ratio 0.003).

The in vitro experiments correlated to some degree with the in vivo analyses, and suggested that components within the urea and creatinine suspensions formed part of the conditioning film. Differences in the film's composition were found after incubation with morning urine compared to afternoon urine. This was not surprising since a previous study had shown that the surface tension of urine differs between people and between sampling times (Reid et al. 1991).

The results illustrated the importance of simulating, where possible, the conditions found in vivo when performing experimental analysis of prosthetic devices. They also documented the adsorption of elements such as calcium and phosphorous, components in encrustations which pose a major problem for long term indwelling devices (Cox et al. 1989; McLean et al. 1988). Not only do encrustations block the flow of urine, they can also develop into infected foci, when pathogens bind to them. This is particularly the case with P. mirabilis, found here in vitro to be highly adherent to the catheters, and known for their production of urease and formation of infected urinary stones (McLean et al. 1988) and toxicity for renal epithelial cells (Mobley et al. 1991).

The bacterial adhesion studies demonstrated the ability of three uropathogens to attach to urinary catheters in buffer and in urinary fluid. For E. coli, the adhesion was 10 fold lower in urine, urea and creatinine (even although there was a 10 fold increase in viable counts due to growth in the urine) than in buffer, suggesting some interference by urinary components in the adhesion process, in agreement with a previous study (Hawthorn and Reid, 1990). This would imply that the E. coli were not binding to carbon-nitrogen, carbon-calcium or carbon-phosphorous containing compounds, as the ratios of these elements increased in the suspending fluids compared to the buffer. The same can be said for the binding of the other two strains.

It is difficult to state from this study what actual substances (proteins, glycoproteins etc) adsorbed from the urine onto the catheter surface. A previous study could not document the binding of the uromucoid Tamm Horsfall protein to catheters (Hawthorn & Reid, 1990). Had this occurred here, the E. coli Hu734 adhesion would likely have been greater than that found using buffer, due to the presence of type 1 fimbriae on the organisms which bind to the mannose residues of the protein.

A previous report has shown that incubation of S. epidermidis with serum coated catheter results in reduced adhesion, (Kristinsson, 1989), and that there was a wide strain-to-strain difference in adhesiveness. In the present study, the S. epidermidis was the least adherent of the three pathogens. As the three strains used here are only representative of their species and of the organisms which cause urinary tract infections, the results should not be generalized for all species of E. coli, S. epidermidis and P. mirabilis uropathogens. Nevertheless, the high adhesiveness of these organisms within only 24 hours in vitro, indicates the potential for catheterized patients to become infected: a fact born out in a study showing that patients with indwelling urethral catheters have a high risk of acquiring a urinary tract infection (Warren et al. 1982).

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Discussion with Reviewers

R. J. C. McLean: How tightly are the urine components bound to the catheter material? Are they easily removed upon washing?

Reid et al.: The nature of the binding was not investigated. When the catheters are rinsed thoroughly in buffer, there are still some traces of elemental adsorption, suggesting some degree of strong binding.

R. J. C. McLean: Do other host components i.e. mucus, bind as well?

Reid et al.: As mentioned in the discussion, the most common uromucoid Tamm Horsfall protein has not yet been shown to bind to the catheter surface. However, it would not be surprising to find that a variety of urinary components, including mucoid glycoproteins, do bind to the surfaces. The identification of molecules within the conditioning film has yet to be undertaken.

B. Ratner: A mechanism by which urea or creatinine solution can deposit a "thick" layer on the catheter is proposed. Urea and creatinine do not polymerize or significantly aggregate in aqueous to form an overlayer film. I propose that bacterial growth is occurring in the medium and you are seeing a bacterial slime film, not a urea or creatinine film. Alternatively, these solutions are extracting the polymer and altering its surface structure. These hypotheses seem reasonable, given the data. Perhaps the reduced bacterial adhesion to all catheters compared to controls is due to an initial bacterial layer that inhibits further bacterial attachment in your in vitro assay?

Reid et al.: The XPS analysis was not done on surfaces treated with urea and creatinine then incubated with bacteria. Thus, the film was not due to bacterial by-products. Also, the strains used here, to our knowledge, are not slime formers.

It is certainly possible that the solutions cause an extraction of elements

from the polymers, thereby altering the surface. However, the polymers are not believed to contain calcium and phosphorous, their oxygen-carbon ratios remained unchanged throughout the experiments, and according to manufacturer publications, they would not be expected to break down within 24 hours. The similar peaks found for urine on glass further supports these conclusions. In the clinical setting, the deposition of substances onto catheters, especially encrustations visible to the human eye, does occur over time. It would seem feasible that the analyses reported here have simply demonstrated this process at it's inception.

The electron micrograph illustrated that within 24 hours, there is adhesion and the commencement of a bacterial monolayer, but there are certainly many sites for other organisms to adhere to. The process of biofilm formation has been well described by others, and on the contrary, the deposition of one layer of organisms often forms the surface to which others adhere. The ability of uropathogens to autoaggregate has also been shown by our group, further supporting the feasibility of bacteria binding to cells already adherent to a catheter. It would seem possible for bacteria to adhere, produce substances to which others cannot adhere, then detach from the catheter surface leaving behind these compounds. However, the adhesion was highest for the PBS samples, so if this type of event is possible for these three pathogens, it was not apparent here. Rather, the reduced adhesion more likely appears to be due to the effects of the host components.