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Methacrylate polymers and copolymers as urinary tract biomaterials: resistance to encrustation and microbial adhesion

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Abstract

The ability of bacteria to adhere to medical device biomaterials within the urinary tract is recognised as an essential mechanism in the pathogenesis of device-related infection. Colonisation of a biomaterial surface with urea-splitting bacteria also causes alkalinisation of the urine with subsequent formation of encrusting deposits of struvite and hydroxyapatite. This study examined the role of both bacterial cell surface hydrophobicity and biomaterial hydrophobicity in urinary device infection and encrustation. The polymers formed and investigated were poly(methyl methacrylate) (PMMA), poly(hydroxyethyl methacrylate) (PHEMA) and copolymers of PMMA with PHEMA (75:25, 50:50 and 25:75 w/w, respectively). Polymer surfaces were characterised by dynamic contact angle measurement in water. Polymer encrustation and adhesion of a hydrophilic *Escherichia coli* urinary isolate and a hydrophobic *Enterococcus faecalis* urinary isolate were also determined. The hydrophobic PMMA polymer was more effective in resisting encrustation than all of the more hydrophilic PMMA/PHEMA copolymer series. Adhesion of the hydrophilic *Escherichia coli* isolate to the copolymers increased with decreasing copolymer hydrophobicity. A relationship was not apparent between copolymer hydrophobicity and adherence of the hydrophobic *Enterococcus faecalis* isolate. © 1997 Elsevier Science B.V.

Keywords: Bacterial adhesion; Encrustation; Poly(methyl methacrylate); Poly(hydroxyethyl methacrylate); Hydrophobicity

1. Introduction

The ability of uropathogens to adhere to the surface of biomaterials is recognised as a mechanism in the initiation and pathogenesis of infection related to devices in the urinary tract. Within hours of attachment bacteria can aggregate and become enclosed in a protective glycocalyx to form a microbial biofilm resistant to antimicrobial therapy and host defences (Tunney et al., 1996b). Colonisation of a biomaterial surface with urea-splitting bacteria also causes alkalinisation of the

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urine, which lowers the solubility of the magnesium salt, struvite and the calcium salt, hydroxyapatite, allowing salt deposition on the device biomaterial. Scanning electron microscopy studies have shown bacterial biofilms on urethral catheters in close association with encrusting materials (Ohkawa et al., 1990; Stickler et al., 1993). For a biomaterial, therefore, to be effective in resisting this type of encrustation it should be able to resist bacterial adhesion and subsequent microbial biofilm formation.

Several series of methacrylate polymers and copolymers have been used to study bacterial adhesion as a function of material hydrophobicity and charge. Hogt et al. (1986) examined adhesion of coagulase-negative staphylococci (CNS) to copolymers formed from different ratios of poly(-methyl methacrylate) (PMMA) and poly(hydroxyethyl methacrylate) (PHEMA). The authors reported that increasing the PHEMA content of the PMMA/PHEMA copolymer resulted in a decrease in hydrophobicity and a decrease in adhesion of the CNS examined. In another study of methacrylate copolymers, Harkes et al. (1991) reported that bacterial adherence was greater on more hydrophobic copolymers. An increase in encrustation resistance has been reported with increasing hydrophobicity of a series of fluorinated copolymers (Holmes et al., 1992). Similarly, encrustation on ureteral stent biomaterials was shown to decrease as hydrophobicity increased (Tunney et al., 1996c).

The aims of this study, therefore, were to synthesise methacrylate polymers and copolymers as potential urinary tract biomaterials/coatings and, to determine the relationship between their hydrophobicity and ability to resist both encrustation and bacterial adherence.

2. Materials and methods

2.1. Materials

Methyl methacrylate monomer (MMA), hydroxyethyl methacrylate monomer and azobisisobutyronitrile (AIBN) were all obtained from Sigma (Dorset, UK).

2.2. PMMA/PHEMA preparation

MMA was washed four times with aqueous sodium hydroxide (2%) to remove the added inhibitor. The lower aqueous layer was discarded upon separation after each wash. The monomer was then washed four times with distilled water, again the aqueous layer being removed on separation. Aqueous washing was continued until no colour change was observed in red litmus paper. The inhibitor-free monomer was then dried using anhydrous magnesium sulphate.

PMMA, PHEMA and copolymers of PMMA with PHEMA (75:25, 50:50 and 25:75 w/w ratios, respectively) were formed by addition of an initiator, AIBN, at a concentration of 0.5% w/w. Moulds for copolymer casting were constructed using two glass plates (200 × 200 mm) separated by rubber tubing of 2 mm thickness on three sides. The plates were held upright and clamped together by G-clamps. The copolymers (20 ml) were then injected into the moulds via the open end of the plates. The moulds were placed in an oven at 60°C for 18 h where the polymerisation reaction took place. On removal from the oven, the copolymers were left to dry for 24 h in a fume cupboard.

2.3. Contact angle measurement

The advancing and receding contact angles of the biomaterials were determined using a CAHN Dynamic Contact Angle Analyser, DCA 312, which was interfaced with a personal computer. For all experiments, a 500-mg tantalum weight was used for calibration and an immersion rate of 150 μm/s was employed. The wetting medium used was high-performance liquid chromatography grade water.

2.4. Encrustation development

Fifteen sections (50 × 10 × 2 mm) of each copolymer were suspended in an artificial urine model and encrusted as described previously (Tunney et al., 1996a). The artificial urine was changed on a daily basis and to simulate conditions in the urinary tract, it was maintained in the

reservoir at a temperature of 37°C and an atmosphere equilibrated with 5% CO_2 (Denyer et al., 1990). Five sections of each copolymer were removed after periods of 2, 6 and 14 weeks for atomic absorption spectroscopy.

2.5. Atomic absorption spectroscopy

Encrusted deposits were dissolved in acetic acid as previously described (Tunney et al., 1996d) and the quantity of magnesium and calcium present was determined by established techniques (Bassett et al., 1978) using a Perkin-Elmer 2380 atomic absorption spectrophotomer. The quantity of magnesium and calcium present on each section was divided by the surface area of that section to give the surface density (mg cm^{-2}) of magnesium and calcium present.

2.6. Adherence of ureteral stent biofilm isolates

Adherence of a hydrophilic *Escherichia coli* isolate and a hydrophobic *Enterococcus faecalis* isolate to copolymer sections was assessed by a radioisotopic method (Bonner et al., 1995). These two ureteral stent biofilm isolates were recovered from microbial biofilm present on retrieved ureteral stents (Keane et al., 1994) and retained within the School of Pharmacy laboratories. The isolates used were maintained on Mueller-Hinton agar (MHA) slopes at 4°C and on blocks of MHA in 100% glycerol at −20°C. Subculturing was performed at 3 monthly intervals.

2.7. Statistical analysis

Statistical analysis was performed using one-way and two-way analysis of variance ($p < 0.05$ denoting significance).

3. Results

Positive correlations were observed between a decrease in the PMMA content of the copolymers and a decrease in the advancing ($r = 0.98$) and receding ($r = 0.97$) contact angles of the copolymers (Fig. 1).

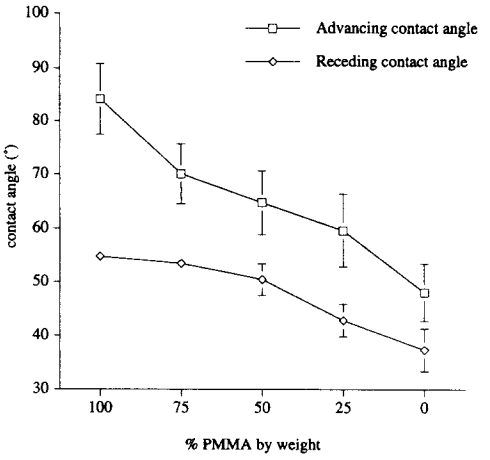

Fig. 1. Variation (mean ± S.D.) in dynamic contact angle with percentage PMMA in PMMA/PHEMA copolymers.

The amount of magnesium and calcium deposited on the surface of each of the copolymers increased with time (Fig. 2(a,b)). In consideration of total encrustation over the 14 week period

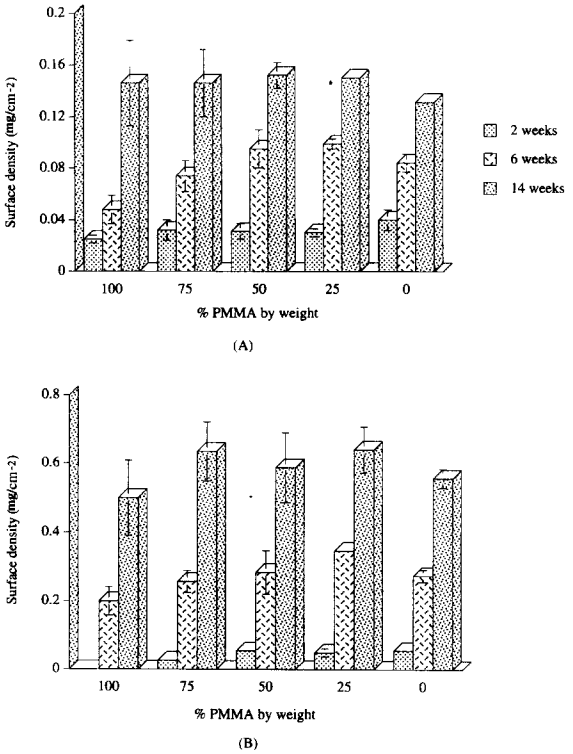

Fig. 2. Variation (mean ± S.D.) of (A) magnesium and (B) calcium deposition with percentage PMMA in PMMA/PHEMA copolymers.

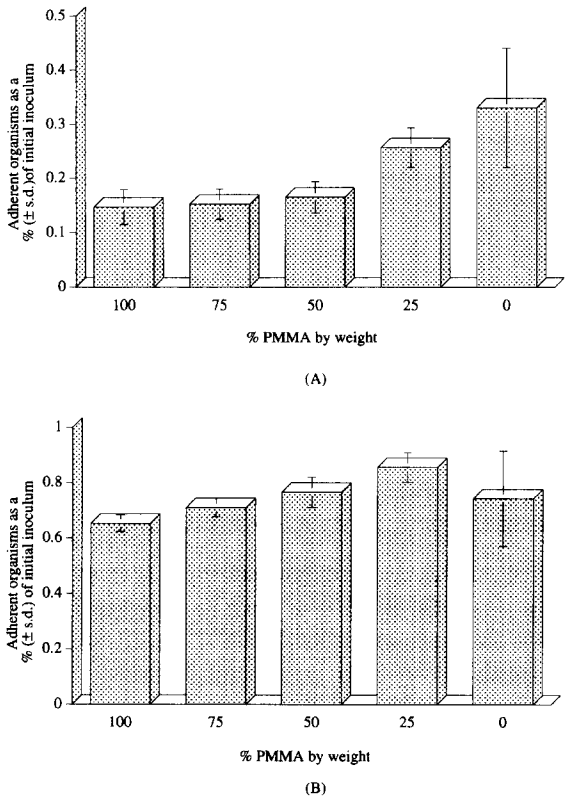

Fig. 3. Variation (mean ± S.D.) of (A) *Escherichia coli* and (B) *Enterococcus faecalis* adherence with percentage PMMA in PMMA/PHEMA copolymers.

sampled for each polymer/copolymer, significantly less encrustation was deposited on the pure PMMA polymer than on any of the other copolymers. No other differences in encrustation deposition on the copolymers were apparent. No correlation was apparent, therefore, between copolymer hydrophobicity and resistance to encrustation. Similarly, as *Enterococcus faecalis* adherence did not differ significantly on the copolymers examined, no correlation was apparent (Fig. 3(b)). In contrast to these findings a correlation ($r = 0.92$) was observed between a decrease in copolymer hydrophobicity and an increase in *Escherichia coli* adherence (Fig. 3(a)).

4. Discussion

The expanding use of urine drainage devices such as catheters, nephrostomy tubes and ureteral stents has been extremely beneficial to patients but has also been associated with unwanted adverse effects. Indwelling ureteral stents have been shown to develop microbial biofilm which may lead to recurrent infection and encrustation on exposure to urine (Gorman et al., 1993). Manufacturers, therefore, currently recommend that ureteral stents should be changed 3 months after insertion in the first instance and, if no encrustation has formed, at 6 month intervals thereafter (Gordon, 1989). The surface properties of the biomaterial used for stent fabrication are thought to be influencing factors with respect to these problems (Gorman, 1995).

Several studies have attempted to relate bacterial adherence to the physicochemical surface properties of the bacteria and the biomaterial surface. Fletcher and Loeb (1979) and Pringle et al. (1983) demonstrated that adhesion of marine bacteria onto glass and various polymers was related to the hydrophobicity of the materials as determined by water contact angles. Bacterial adherence was reported to be maximal on materials with water contact angles between 65° and 85° and to decrease on materials having contact angles on either side of this range. Minagi et al. (1985) reported differences in the adhesion of *Candida* species to denture base resin materials differing in hydrophobicity. A hydrophobic *Candida tropicalis* strain adhered preferentially to hydrophobic materials, whereas a hydrophilic *Candida albicans* strain adhered preferentially to hydrophilic materials. The authors concluded that adherence was maximal when the surface free energy of the materials was closer to that of the microorganisms and decreased when materials had higher or lower surface free energies than that of the microorganisms. In an attempt to determine the effect of material surface character on bacterial adherence, Hawthorn and Reid (1990) examined adherence of *Escherichia coli*, *Staphylococcus epidermidis* and *Lactobacillus acidophilus* to a range of biomaterial surfaces differing in hydrophobicity. Water contact angle measurements demonstrated that *Escherichia coli* was more hydrophobic than *Staphylococcus epidermidis* and *Lactobacillus acidophilus*. Both *Lactobacillus acidophilus* and *Staphylococcus epidermidis*

showed much greater adherence to each biomaterial examined than *Escherichia coli*. *Lactobacillus acidophilus* was the only isolate to show any correlation between biomaterial surface tension and adherence. This organism tended to bind preferentially to more hydrophobic materials (low surface free energy) than hydrophilic materials (high surface free energy). Similarly, in an examination of *Streptococcus sanguis* adhesion to polymers with different surface free energies, Van Pelt et al. (1985) reported that the highest number of bacteria was found on polytetrafluoroethylene which was hydrophobic and the lowest was found on glass which was hydrophilic.

Differences in bacterial adhesion have also been reported with respect to methacrylate polymers and copolymers differing in hydrophobicity. Hogt et al. (1986) reported that adhesion of CNS to a series of PMMA/PHEMA copolymers decreased with decreasing hydrophobicity of the copolymers. Similarly, Harkes et al. (1991) reported that three strains of *Escherichia coli* adhered preferentially to hydrophobic methacrylate copolymers.

In contrast to the results of these studies, others have failed to correlate biomaterial hydrophobicity and bacterial adherence. For example, Oga et al. (1988) reported that adhesion of *Staphylococcus epidermidis* was greater to PMMA than to polyethylene, despite the fact that the two biomaterials had similar hydrophobicities. Similarly, Boulange-Petermann et al. (1993) found no correlation between adhesion of *Streptococcus thermophilus* and the hydrophobicity of stainless steel surfaces. In a more recent study, Cormio (1995) reported that bacterial adherence to a range of double-J stents did not differ significantly. The authors did, however, find that there were statistically significant differences in the adhesion capacities of the bacterial strains tested, leading the author to conclude that the adhesive properties of the bacteria are more important than the properties of the biomaterial used for stent manufacture.

In the present study, a homologous series of methacrylate polymers and copolymers were formed with differing hydrophobicities. A correlation was found between decreasing PMMA content of the copolymers and decreasing hydrophobicity as determined by water contact angle measurement. Adhesion of the hydrophilic *Escherichia coli* strain increased with decreasing hydrophobicity of the copolymers. This result is similar to that reported by Minagi et al. (1985) who found that adhesion of a hydrophilic *Candida albicans* strain increased with decreasing hydrophobicity of denture base resin. However, it contrasts with the results reported by Hogt et al. (1986), who showed that adhesion of both hydrophilic and hydrophobic CNS decreased with decreasing biomaterial hydrophobicity. The results also contrast with those reported by Harkes et al. (1991) who showed that adhesion of three strains of *Escherichia coli* decreased with decreasing biomaterial hydrophobicity. Unfortunately, the authors did not report on the hydrophobic nature of the strains used and, therefore, the results of their study cannot be properly compared with the results of the present study.

The hydrophobicity of biomaterial surfaces has also been shown to affect their ability to resist encrustation. Holmes et al. (1992) examined the in vitro encrustation of a series of fluorinated copolymers and found that as fluorination of the polymer increased, encrustation on its surface decreased. The authors linked the observed decreased encrustation to an increase in the hydrophobicity and hence a decrease in the surface free energy of the fluorinated copolymers. We have previously shown that encrustation on the surface of ureteral stents decreased as the hydrophobicity of the biomaterial surface decreased (Tunney et al., 1996c). In the present study, the most hydrophobic polymer, pure PMMA, was more effective at resisting encrustation than any of the other copolymers studied. However, as the hydrophobicity of the copolymers decreased with decreasing PMMA content, there were no further significant differences in encrustation resistance.

This study has shown that for this series of homologous copolymers there was no relationship between hydrophobicity and encrustation resistance. However as copolymer hydrophobicity decreased, adherence of a hydrophilic *Escherichia coli* increased suggesting that within the urinary tract, colonisation of a biomaterial surface with certain urinary pathogens is dependant on the surface hydrophobicity of both the biomaterial

and the pathogen. This highlights the difficulties encountered in developing novel biomaterials resistant to bacterial attachment and encrustation in the urinary tract.

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