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Published in:
Molecular Pharmaceutics

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

An Infection-Responsive Approach to Reduce Bacterial Adhesion in Urinary Biomaterials

Colin P. McCoy*, Nicola J. Irwin, Christopher Brady, David S. Jones, Louise Carson, Gavin P. Andrews, Sean P. Gorman

School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK.

*To whom correspondence should be addressed. (Tel: +44 28 9097 2081; Fax: +44 28 9024 7794; e-mail: c.mccoy@qub.ac.uk)

Abstract

Infection is an inevitable consequence of chronic urinary catheterisation, with associated problems of recurrent catheter encrustation and blockage experienced by approximately 50% of all long-term catheterised patients. In this work we have exploited, for the first time, the reported pathogen-induced elevation of urine pH as a trigger for 'intelligent' antimicrobial release from novel hydrogel drug delivery systems of 2-hydroxyethyl methacrylate and vinyl-functionalised nalidixic acid derivatives, developed as candidate infection-resistant urinary catheter coatings. Demonstrating up to 20-fold faster rates of drug release at pH 10, representing infected urine pH, than at pH 7, and achieving reductions of up to 96.5% in *in vitro* bacterial adherence, our paradigm of pH-responsive drug delivery, which requires no external manipulation, therefore represents a promising development towards the prevention of catheter-associated urinary tract infections (CAUTIs) *in vivo*.

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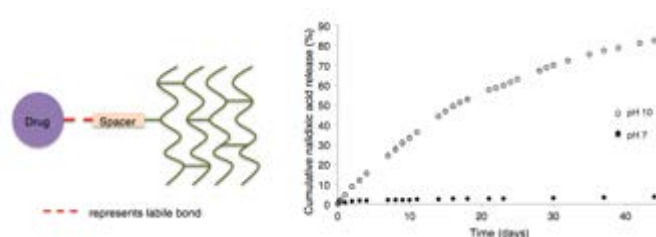
Keywords

pH-Responsive; Urinary catheter infection; Ester hydrolysis; Drug conjugate copolymer

Introduction

The employment of biomaterials in diverse diagnostic and therapeutic applications has led to significant advances within the practice of modern medicine, (1) however, clinical applicability is ultimately limited by their inherent susceptibility to bacterial colonisation. As one of the most predominant biomaterial-associated infections, catheter-associated urinary tract infections (CAUTIs) deserve particular attention. (2) Complications primarily result from activity of the bacterial enzyme, urease, which is secreted by many urinary pathogens, of which *Proteus mirabilis* is the most notorious. The resulting urease-catalysed hydrolysis of urea into ammonia elevates urinary pH to reported values up to pH 9.1, thereby triggering precipitation of magnesium and ammonium phosphate crystals. (3) Complications, including crystalline biofilm formation, catheter encrustation and blockage, are experienced on a recurrent basis by approximately 50% of all long-term catheterised patients. (4)

Complete eradication of biomaterial-associated infections is difficult due to the recalcitrance of bacteria in the biofilm-mode of growth, therefore much emphasis has now been placed on preventing infections by modification of the device to resist bacterial attachment. Strategies investigated to-date include functionalisation of the surface with bacteria-repelling polymer brushes, (5) use of bioinert multilayer polymer coatings (6) and loading the device with antibacterial agents. Passive, diffusion-controlled release of drug from the matrix interior of currently marketed antimicrobial biomaterials represents a suboptimal release strategy. The antimicrobial lifetime of the device is restricted by an initial burst of surface-localised agent, which is thereafter followed by continuous drug elution in subtherapeutic levels, potentially promoting the emergence of bacterial resistance. (7) One strategy to control release kinetics and ultimately achieve tailored drug delivery with a precise 'on-off' response, (8) while also mitigating the compromise in mechanical properties associated with particulate release from the matrix interior, (9) involves the application of labile drug conjugates to three-dimensional drug delivery systems. (10, 11)

We have recently described the synthesis and pH-responsive release kinetics of a suite of novel, polymerisable drug conjugates containing the quinolone antimicrobial agent, nalidixic acid, covalently conjugated *via* hydrolytically labile ester bonds to alkenyl spacer moieties of varying vinyl chain lengths. (12) Of particular significance towards the development of pH-responsive, infection-resistant urinary biomaterials is the faster hydrolysis and subsequent release of nalidixic acid from these conjugates at rates up to 17-fold greater at pH 9.29 than at pH 7. These ester derivatives of nalidixic acid were herein copolymerised, for the first time, with 2-hydroxyethyl methacrylate (2-HEMA), employing ethyleneglycol-dimethacrylate (EGDMA) as a crosslinker, to develop three-dimensional chemically-triggered drug delivery systems. Characterisation of the synthesised copolymers in terms of water wettability, swelling characteristics, pH-mediated release kinetics, and resistance to adherence of *P. mirabilis* and *Staphylococcus aureus* informs the use of these drug conjugate-HEMA copolymers as infection-resistant catheter coatings capable of exploiting the elevation of urine pH reported at the onset of CAUTIs by urease-producing urinary pathogens to trigger release of the conjugated antimicrobial agent, specifically through controlled ester hydrolysis.

Experimental Section
Materials
Polymerisable ester nalidixic acid conjugates: 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 2-propen-1-ol ester **1**, 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 3-buten-1-ol ester **2**, and 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 9-decen-1-ol ester **3**, the molecular structures of which are displayed in Figure 1, were synthesised as previously described. (12)

2-Hydroxyethyl methacrylate (2-HEMA), ethyleneglycol-dimethacrylate (EGDMA), phosphoric acid, acetic acid, boric acid, hydrochloric acid, potassium chloride and sodium hydroxide were purchased from Sigma-Aldrich (Poole, Dorset, UK). 2,2'-Azobisisobutyronitrile (AIBN) was obtained from VWR International Ltd. (Lutterworth, UK). Phosphate-buffered saline (PBS), tryptone soya broth (TSB), quarter-strength Ringer's solution (QSRS) and Mueller-Hinton broth (MHB) were obtained from Oxoid Ltd (Hampshire, UK). PBS and TSB were adjusted to pH 10 by addition of sodium hydroxide.

Proteus mirabilis ATCC 35508 and *Staphylococcus aureus* ATCC 6538 (LGC Standards, Middlesex, UK) were maintained on cryopreservative beads (Protect Bacterial Preservation System, Technical Service Consultants Ltd., UK) in 10% glycerol at -80°C and cultivated in MHB at 37°C when required for the microbiological assessments.
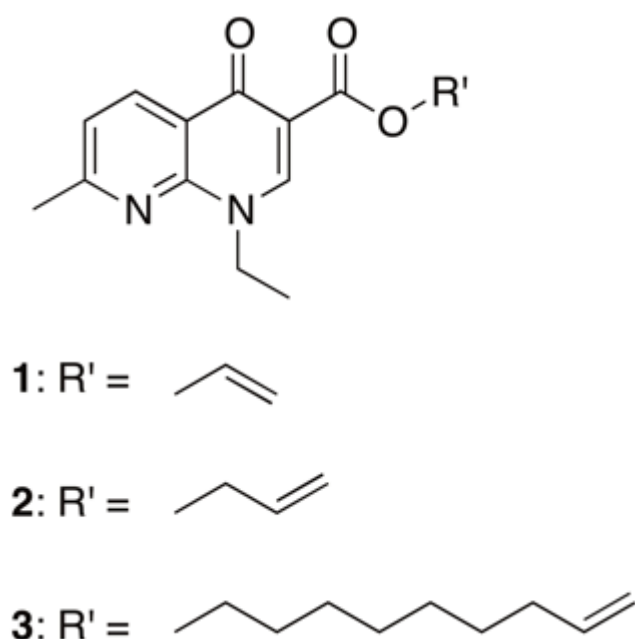

Figure 1: Molecular structures of polymerisable ester drug conjugates: 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 2-propen-1-ol ester **1**; 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 3-buten-1-ol ester **2**; and 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 9-decen-1-ol ester **3**.

Methods

Synthesis of Poly(2-Hydroxyethyl Methacrylate) (p(HEMA)) Homopolymers

Films of p(HEMA) were synthesised, as previously described, (13) by dissolving EGDMA (1% w/w) and AIBN (1% w/w) (crosslinker and initiator, respectively) at ambient temperature in 2-HEMA (98% w/w), with stirring. The total mass of polymer was 10 g. The resulting solution was injected slowly into moulds comprising two glass plates, which were separated with medical grade silicone tubing with lumen diameter of 0.3 mm and wall thickness of 0.18 mm, lined with release liner, and clamped using spring clips. Following polymerisation at 90°C for a period of 3 h, the formed films were soaked for one week in deionised water to remove unreacted monomers and initiator, then dried to constant weight in an oven at 60°C to form xerogels. The removal of all monomers from the films was verified by monitoring acrylate absorption in the rinsing media at 220 nm.

Synthesis of Nalidixic Acid Conjugate-HEMA Copolymers

Copolymers **4**-**6** were synthesised by stirring the respective conjugate from **1**-**3** (5% w/w) with EGDMA (1% w/w) and AIBN (1% w/w) at ambient temperature with 2-HEMA (93% w/w). The resulting solution was injected slowly into moulds, as described for p(HEMA), then polymerised at 90°C for one week. In all cases, total copolymer mass was 10 g. The formed copolymers were soaked for three weeks in deionised water to remove unreacted monomers.

Contact Angle Analysis

Sessile contact angles of the surfaces of the p(HEMA) homopolymer and the drug conjugate-HEMA copolymers **4**-**6** were determined using a First Ten Ångströms (FTA) 200 video-based contact angle analyser (Portsmouth, Virginia, USA) in conjunction with FTA 32 video software for image capture and analysis. The tangent associated with a single drop of deionised water, used as the wetting medium, was measured in the equilibrium position on the sample surface. (14, 15)

Equilibrium Swelling Studies

The equilibrium degrees of swelling of p(HEMA) and the drug conjugate-HEMA copolymers **4**-**6** were examined in pH 7 universal buffer, prepared according to the formulation of Koller *et al.* (1992) (16) reported in Table 1. Replicate discs (1 cm^2) were dried in an oven at 60°C until constant weight, weighed, and placed in pH 7 universal buffer. At designated intervals, discs were removed, blotted with filter paper to remove excess surface water, and reweighed, as previously described. (17) Discs were returned to the buffer media and soaked until no further change in mass. The weight percentage of buffer within the swollen polymer, designated as the equilibrium water content (EWC), was calculated according to the following equation (18) :

$$EWC\ (\%) = (M_f - M_i)/M_i \times 100$$

where M_f and M_i represent the final sample mass after water absorption and the initial dried sample mass respectively.

Table 1: Formulation of Universal Buffer

pH	Stock (μL)[a]	2 M NaOH (μL)	KCl (mg)	H$_2$O (mL)
7	833	437	25.8	11.43
10	714	557	0	11.44

[a]Stock solution (100 mL) contained 2.7 mL H$_3$PO$_4$, 2.29 mL AcOH and 2.48 g H$_3$BO$_3$ dissolved in deionised H$_2$O. (16)

In Vitro pH-Mediated Drug Release Kinetics

Kinetics of nalidixic acid release from the drug conjugate-HEMA copolymers **4-6** were examined at pH 7 and pH 10 in universal buffer solutions with constant ionic strength, prepared according to the formulation of Koller *et al.* (1992) (16) reported in Table 1. Pre-swollen copolymer discs (1 cm^2), suspended on a needle, were placed in McCartney bottles containing 10 mL pre-warmed universal buffer solution of the appropriate pH and shaken at 37°C in an oscillating water bath (100 rpm). At predetermined intervals, the discs were transferred to fresh media (10 mL, to ensure sink conditions) and the released drug quantified by UV-visible spectroscopy (λ = 257 nm), with concentration dependence of nalidixic acid on absorbance determined from calibration curves prepared using universal buffer of the appropriate pH ($r^2 >$ 0.999), as previously described. (19) Release experiments at each pH were performed in quintuplicate.

In Vitro Microbiological Analysis

Bacterial suspensions of *P. mirabilis* and *S. aureus* (1 x 10^8 cfumL^{-1}), in either PBS (pH 7) supplemented with 0.5% TSB (pH 7), or PBS (pH 10) supplemented with 1% TSB (pH 10), were diluted 1 in 100 with PBS containing 0.5% TSB of the required pH. Replicate discs (8 mm) of copolymers **4-6** and p(HEMA) (as control) were cut from the polymer films, placed in individual wells of a 24-well flat bottom tissue culture plate (Corning Inc., Corning, NY), and completely immersed with aliquots of the bacterial suspension (1 mL) with density of 1 x 10^6 cfumL^{-1}. The plates were continuously shaken in an orbital incubator at 37°C. After designated time intervals of 4 h and 24 h, the discs were removed from the bacterial suspension using sterile forceps, and non-adherent bacteria removed by washing for 30 sec in QSRS (3 x 5 mL). (20) The discs were transferred into fresh QSRS (5 mL), sonicated for 10 min, and vortexed for 30 s to remove adherent bacteria. A viable count of the QSRS was performed by the Miles and Misra serial dilution technique, (21) followed by plating onto low-swarm (LSW) agar (*P. mirabilis*) or Mueller-Hinton agar (*S. aureus)* to enumerate the previously adhered bacteria per disc. The sonication technique has previously been demonstrated not to affect bacterial viability or morphology. (22)

Statistical Analysis

Statistical differences between the surface contact angles and equilibrium water contents of copolymers **4-6** and the p(HEMA) homopolymer were evaluated by a one-way analysis of variance, whereas the effect of pH and conjugate alkyl chain length on the cumulative amounts of nalidixic acid released at designated intervals, and the effect of pH and copolymerisation with the ester drug conjugates on bacterial adherence after 4 h and 24 h were statistically evaluated by a two-way analysis of

variance, followed by Tukey's honestly significant difference test for post-hoc comparisons between means of individual groups. In all cases, differences were considered significant when $p < 0.05$. (23)

Results and Discussion

The effect of copolymerisation with the drug conjugates on surface wettability and swelling characteristics was herein examined to assess changes in surface hydrophilicity/hydrophobicity and resulting water contents. These surface properties not only play a role in controlling rates of drug release, but are also involved in determining ultimate device performance *in vivo*. Application of the synthesised copolymers as medical device coatings provides a useful mechanism of modifying surface properties to optimise resistance to bacterial colonisation, improve device biocompatibility and minimise frictional irritation during device insertion and removal, whilst retaining bulk mechanical properties. (10, 11)

Contact Angle Analysis

The water wettability of the nalidixic acid conjugate-HEMA copolymers **4**-**6**, prepared from the respective conjugates **1**-**3** (5% w/w), 2-HEMA (93%), and EGDMA crosslinker (1% w/w), was assessed in comparison to the control p(HEMA) homopolymer by measuring the static contact angles of deionised water on the sample surfaces. The contact angles measured for the materials are presented in Table 2.

Table 2: Static Contact Angles of p(HEMA) and Nalidixic Acid Conjugate-HEMA Copolymers

Polymer	Contact Angle (°) (Mean ± S.D.)
p(HEMA)	52.5 ± 3.86
4	75.6 ± 3.44
5	80.1 ± 3.25
6	61.5 ± 2.63

Copolymerisation with the ester drug conjugates **1**-**3** (5% w/w) caused a significant increase in surface contact angle relative to the p(HEMA) homopolymer, as reported in Table 2. This observed reduction in water wettability of the material surfaces was most likely attributed to the partial replacement of hydrophilic OH groups of p(HEMA) with comparatively more hydrophobic CH moieties of the conjugate alkyl chains.

In contrast to the expected effect of alkyl chain length on water wettability of the surface, (24) post-hoc analysis herein revealed that the surface of the longer-chain conjugate copolymer **6** was significantly more hydrophilic, as inferred by the statistically lower contact angle (61.5°), than the surfaces of the shorter-chain conjugate copolymers **4** and **5** (75.6° and 80.1°, respectively). This observation was related to the higher relative molecular mass of conjugate **3** and the resultant lower molar ratio of amphiphilic nalidixic moieties within copolymer **6** than within copolymers synthesised from conjugates **1** or **2**. Relative to p(HEMA), the surface of **6** was consequentially modified to a lower extent than the surfaces of the shorter-chain conjugate copolymers **4** and **5**. In accordance with previous reports, (25) differences in the surface orientation of the amphiphilic nalidixic moiety, namely

closer proximity to the surfaces of the short-chain conjugate copolymers **4** and **5** than the surface of copolymer **6**, as a result of outward projection of this functional group by the longer alkyl chains of the latter, furthermore, contributed to the greater reduction in surface hydrophilicity observed with copolymers **4** and **5**.

Equilibrium Swelling Studies
The equilibrium water contents (EWCs) of the drug conjugate-HEMA copolymers **4**, **5** and **6** in comparison to the p(HEMA) homopolymer, recorded after immersion in pH 7 universal buffer for 72 h, after which no further weight increase was recorded, are reported in Table 3.

Table 3: Equilibrium Water Contents of p(HEMA) and Nalidixic Acid Conjugate-HEMA Copolymers

Polymer	EWC (%) (± S.D.)
p(HEMA)	75.6 ± 0.76
4	74.0 ± 0.90
5	75.1 ± 1.07
6	70.3 ± 0.58

Copolymer **6**, synthesised from 2-HEMA and the longer-chain conjugate **3**, displayed a statistically lower EWC than the p(HEMA) homopolymer and short-chain conjugate copolymers **4** and **5** (Table 3). Polymer swelling is dependent on properties of the swelling medium, such as pH, temperature and ionic strength, and the polymer itself, such as the hydrophilic/hydrophobic balance, crosslink density and the presence of ionisable functional groups. (26) In contrast to the constant universal buffer and degree of crosslinking (1% w/w), one factor that was comonomer-dependent and played a predominant role in the significant difference in EWC between p(HEMA) and copolymer **6** observed herein was the hydrophilic/hydrophobic balance of the polymer networks. As previously reported, (27) the more hydrophobic nature of the longer-chain conjugate **3** in comparison to 2-HEMA, **1** and **2** was responsible for reducing the overall polymer wettability and weakening the subsequent interactions between the copolymer network and the aqueous swelling medium. In addition, chain entanglements of the long alkyl chain of conjugate **3** within the copolymer effectively interrupted relaxation of the polymer network and thereby reduced the resultant degree of swelling.

In Vitro **pH-Mediated Nalidixic Acid Release Kinetics**
The theory behind the development of these three-dimensional chemically-conjugated drug delivery systems as candidate infection-resistant catheter coatings is that upon elevation of urinary pH, hydrolysis of the ester bond linking nalidixic acid to the alkyl spacer moiety will be triggered with immediate effect, allowing free drug to effectively target biofilm bacteria at their fulcrum for adherence, before secretion of the protective glycocalyx. To investigate the pH-responsive properties of these ester drug conjugate-HEMA copolymers, the rate of nalidixic acid release from copolymers **4**-**6** was characterised as a function of pH. Furthermore, the concept of tailoring rates of release *via* the employment of spacers of varying vinyl chain lengths was explored. Indwelling catheters may remain *in situ* for up to six weeks between scheduled changes, therefore release studies were continued for >42 days to assess ester bond

stability during typical periods of device implantation. The *in vitro* pH-mediated kinetics of nalidixic acid release from copolymers **4-6** are displayed in Figure 2.
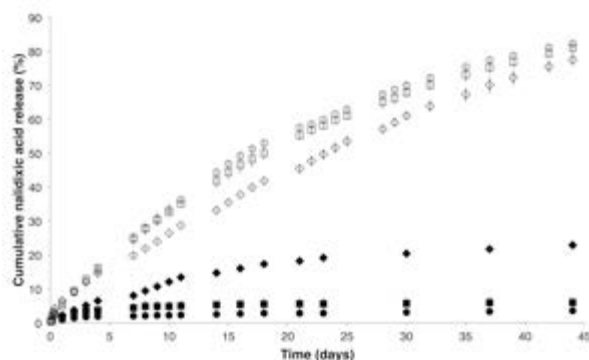

Figure 2: The effect of pH on the mean (± S.D.) release of nalidixic acid from copolymers **4**, **5** and **6**, represented by *circles*, *squares* and *diamonds,* respectively, at 37°C with shaking. Sink conditions were maintained throughout the study. *Open* and *closed symbols* refer to release at pH 10 and pH 7 respectively.

All copolymers displayed the same general trend in terms of release rate with respect to pH of the dissolution media. The ester bond mediating attachment of nalidixic acid to the alkyl spacer moiety was relatively stable at normal physiological urine pH, pH 7, (28, 29) and therefore successfully delayed drug release from the copolymers, in contrast to the significantly more rapid rates of release demonstrated at the elevated pH typical of CAUTIs, pH 10, as shown in Figure 2. This strategy is therefore of major applicability towards the development of infection-responsive urinary catheter coatings. As a representative example, cumulative release of nalidixic acid after one week from copolymer **4** was 12-fold greater at pH 10 than at pH 7; cumulative amounts of nalidixic acid released were 24.6% and 1.99%, respectively. We have previously reported the rapid diffusion-controlled release of nalidixic acid from physically-loaded p(HEMA) matrices, where more than 90% of the loaded nalidixic acid was released from the hydrogels within one hour of immersion in media of pH 7 and pH 9. (13) Release of nalidixic acid from the synthesised copolymers described herein, in contrast, approximated 80% after six weeks in media of pH 10, confirming the mechanism of release as hydrolysis-controlled. The increase in release kinetics as the concentration of hydroxide ions increased was attributed to basic catalysis of the hydrolysis reaction, as previously reported. (30)

In addition to environmental conditions, hydrolysis of polymer-drug conjugates is dependent on factors such as the nature and stability of the hydrolysable bond, the physical and chemical composition of the polymer, and steric overcrowding at the cleavage site, (31) however, conflicting reports regarding the relative importance of these factors are found. (32, 33) Of particular relevance to this *in vitro* study is the previously reported faster liberation of acyclovir, *via* ester hydrolysis, from polymer prodrugs with longer spacer moieties between the drug and the main polymer chain, attributed to the reduced steric hindrance for the incoming hydrolytic species. (34) The effect of conjugate alkyl chain length on subsequent release kinetics in this study was, however, less evident, particularly in buffer of pH 10. In contrast to the expected faster hydrolysis and subsequent release of nalidixic acid from the longer-chain conjugate copolymer **6** than from the shorter-chain conjugate copolymers **4** and **5**, as was observed at pH 7 (cumulative release from copolymers **4**, **5** and **6** approximated 1.99%, 4.42% and 8.02% respectively after one week),

cumulative amounts of nalidixic acid released from **6** were lower than from **4** and **5** from day three onwards at pH 10, following release of the surface-localised nalidixic acid. For example, after one week, cumulative release approximated 25.1%, 24.6% and 19.8% from copolymers **4**, **5** and **6**, respectively. Reduced significance of ester bond accessibility to rates of drug release in media of alkaline pH has previously been attributed to the higher ester reactivity at elevated pH. (30) Furthermore, the comparatively lower rates of nalidixic acid release from copolymer **6** than from the shorter-chain conjugate copolymers **4** and **5** at pH 10 was a combined result of the more tortuous copolymer matrix and the associated increase in hydrophobicity within the vicinity of the ester bond upon employment of longer alkyl spacers, as has been previously reported. (10, 27)

Importantly, to effectively prevent bacterial colonisation, concentrations of drug in the release phase must exceed those required for inhibitory and cidal effects against the respective pathogens. This was assessed by *in vitro* microbiological studies and is reported below.

In Vitro Microbiological Analysis

To establish the utility of these chemically-triggered drug delivery matrices as biofilm-resistant urinary catheter coatings, the efficacy of the copolymers in resisting adherence of *P. mirabilis* and *S. aureus* relative to the p(HEMA) homopolymer was assessed herein by challenging samples of **4**, **5**, **6** and p(HEMA) with inoculi of 1 x 10^6 cfumL^{-1} in media of pH 7 and pH 10 over time periods of 4 h and 24 h. Adherence of *P. mirabilis* to the drug conjugate-HEMA copolymers relative to the control p(HEMA) homopolymer is displayed in Figure 3.
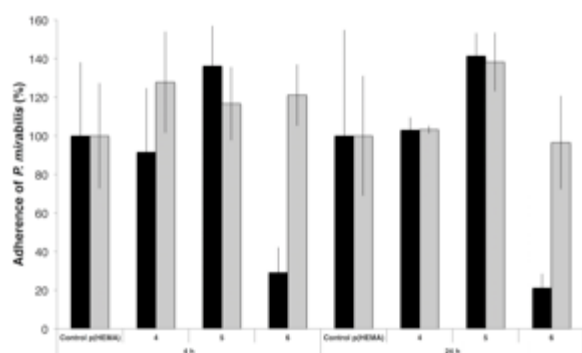

Figure 3: Microbial adherence (%) of *P. mirabilis* to unmodified p(HEMA) and copolymers of 2-HEMA and conjugates **1**, **2** and **3**, relative to the p(HEMA) homopolymer, denoted control, after 4 h and 24 h incubation at 37°C in media of pH 7 (*black*) and pH 10 (*grey*). Columns and error bars represent means ± S.E. (*n* = 5).

The greatest reductions in bacterial adherence relative to the p(HEMA) control were achieved by copolymer **6** in media of pH 7, with adherence reduced by mean values of 70.9% and 78.9% after 4 h and 24 h, respectively (Figure 3). Critical concentrations of nalidixic acid required for inhibitory and cidal effects against *P. mirabilis* have previously been determined as 9.38 mgL^{-1} and 37.50 mgL^{-1} (pH 7), and 26.04 mgL^{-1} and 83.33 mgL^{-1} (pH 10), respectively. Consequential to the low initial loading of the drug conjugate monomers, the mean concentrations of nalidixic acid released from discs (1 cm^2) of **4**, **5** and **6** in pH 7 buffer after 24 h were 0.29 mgL^{-1}, 0.50 mgL^{-1} and 2.23 mgL^{-1}, respectively. Furthermore, in pH 10 buffer, when release was most rapid, cumulative release was again too low: mean concentrations of nalidixic acid were 1.59 mgL^{-1}, 1.59 mgL^{-1} and 6.75 mgL^{-1} after 24 h incubation of

discs (1 cm^2) of **4**, **5** and **6**, respectively. *In vivo*, however, concentrations are dependent on the site of implantation; in contrast to the relatively high urinary turnover rates experienced by ureteral stents as a result of the constant flow of urine from the kidneys, Foley catheters are subjected to comparatively lower turnover rates. Moreover, the retention balloon and the section of catheter immediately above the balloon reside within the bladder and are consequentially exposed to an essentially stagnant pool of urine. (35)

The differences in bacterial adherence observed, for example, between copolymer **6** and the p(HEMA) control were therefore caused by changes in the physicochemical properties of the material surface, namely the reduction in surface wettability after copolymerisation with conjugate **3** in combination with the outward projection of the alkyl chains of **3** away from the copolymer surface, which ultimately make the surface less attractive to bacterial attachment.

The comparative resistances of the nalidixic acid conjugate-HEMA copolymer and p(HEMA) homopolymer surfaces to adherence of *S. aureus* at pH 7 and pH 10 are displayed in Figure 4.
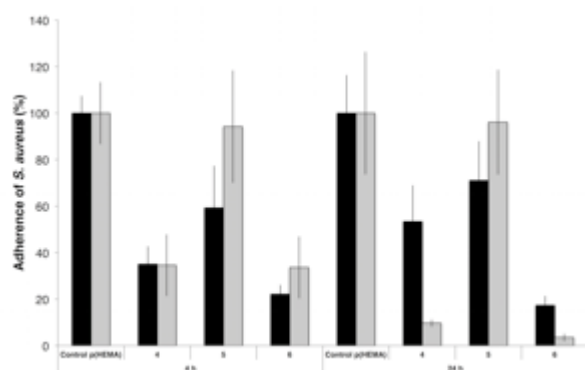

Figure 4: Microbial adherence (%) of *S. aureus* after 4 h and 24 h to unmodified p(HEMA) and copolymers of 2-HEMA and conjugates **1**, **2** and **3**, relative to the p(HEMA) homopolymer, denoted control, after 4 h and 24 h incubation at 37°C in media of pH 7 (*black*) and pH 10 (*grey*). Columns and error bars represent means ± S.E. (*n* = 5).

After 4 h contact with the *S. aureus* challenge, significant reductions in bacterial adherence to copolymer **6** relative to the p(HEMA) homopolymer were demonstrated, irrespective of pH, with adherence reduced by mean values of 77.8% and 66.3% at pH 7 and pH 10, respectively. These significant reductions in bacterial adherence to copolymer **6** relative to the p(HEMA) homopolymer were maintained after 24 h contact with the bacterial challenge. Bacterial adherence to copolymer **4** after 24 h in pH 10 media was also significantly reduced relative to the p(HEMA) homopolymers suspended in pH 7 and pH 10 media.

Again these differences in bacterial adherence to the copolymers relative to the p(HEMA) control were attributed to factors other than the bacteriostatic or bactericidal activities of nalidixic acid due to the low amounts of nalidixic acid released from the copolymers and the well-established poor activity of this quinolone antibacterial agent against *Staphylococci* spp.. MIC values of nalidixic acid against *S. aureus* have previously been determined as 23 mgL^{-1} and 1000 mgL^{-1} at pH 7 and pH 10, respectively, whereas no cidal activity was demonstrated against this isolate at either pH value when concentrations up to 2000 mgL^{-1} were tested. The observed

reductions in bacterial adherence were therefore again attributed to changes in surface wettability and the presence of surface-localised alkyl chains following copolymerisation with the respective drug conjugates.

Despite a low initial conjugate loading of 5% w/w within the copolymer, an encouraging, greater than one-logarithmic reduction in adherence of *S. aureus* was achieved by copolymer **6** relative to the p(HEMA) control after 24 h in pH 10 media. Tuning of the drug conjugate loading within the copolymers to promote greater release of nalidixic acid from the surface is therefore expected to ultimately enhance the efficacy of the synthesised copolymers in resisting bacterial adherence relative to unmodified p(HEMA).

Conclusions

The smart polymeric prodrug matrices developed herein as candidate infection-responsive drug delivery systems are capable of releasing antimicrobial agents specifically, and rapidly, upon elevation of pH to values typically reported at the onset of urinary catheter infections. All nalidixic acid conjugate-HEMA copolymers clearly demonstrate triggered release profiles with negligible release at pH 7, in the absence of urease-producing pathogens, and up to 20-fold faster rates of release upon elevation of pH to pH 10, as occurs *in vivo* due to the urease-catalysed hydrolysis of urea to ammonia. Initial microbiological assessment of copolymers containing a 5% w/w loading of drug conjugate suggests that application of the conjugate-HEMA copolymers as urinary catheter coatings is a promising strategy towards the prevention of CAUTIs *in vivo*.

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