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Characterization of crosslinking effects on the physicochemical and drug diffusional properties of cationic hydrogels designed as bioactive urological biomaterials

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Abstract

This study examined the effects of concentration and type of crosslinker (tetraethyleneglycol diacrylate, TEGDA; diethyleneglycol dimethacrylate, DEGDMA; and polyethyleneglycol dimethacrylate, PEGDMA) on the mechanical and drug diffusional properties of hydrogels that had been selected as candidate coatings for bioactive medical devices. Hydrogels (dimethylaminoethylmethacrylate-co-vinylpyrrolidone; 1:1) were prepared by free radical polymerization and characterized using tensile analysis, dynamic contact angle analysis and analysis of swelling at pH 6.0. The release of fusidic acid and chlorhexidine was evaluated using buffered medium at pH 6.0 and, in addition, using dissolution medium that had been buffered to pH 9 in the presence and absence of elevated concentrations of calcium, representative of urinary encrustation. Crosslinker concentration, but not type, affected the advancing and receding contact angles. Conversely, both crosslinker type and concentration affected the mechanical and swelling properties of the hydrogels. Maximum swelling and elongation at break were associated with the PEGDMA-crosslinked hydrogels whereas TEGDA-crosslinked hydrogels exhibited the maximum ultimate tensile strength and Young's modulus. Drug release from all systems occurred by diffusion. The mass of chlorhexidine and fusidic acid released was dependent on crosslinker type and concentration, with hydrogels crosslinked with PEGDMA offering the greatest mass of drug released at each sampling period. The mass of fusidic acid but not chlorhexidine released at pH 9.0 in a calcium augmented medium was lower than that released in the same medium devoid of elevated calcium, due to the formation of the poorly soluble calcium salt. In conclusion, this study has uniquely examined the effects of crosslinker type and concentration on physicochemical and drug release properties essential to the clinical and non-clinical performance of bioactive hydrogels for medical device application.

Introduction

Medical device-related infection is a problem that is common to all medical devices and is associated with considerable morbidity and mortality (Jones et al 2002b, 2003; Hanlon et al 2004). Whilst research is ongoing to develop polymer surfaces that offer resistance to microbial adherence (and hence medical device-related infection), it is accepted that the resistance of medical devices to microbial adherence may be further enhanced by the use of bioactive biomaterials (Raad & Hanna 1999; Gorman & Jones 2002, 2003; Birkenhauer et al 2004). Following their introduction into biological fluids, the antimicrobial agent is released and, providing the rate of drug release is sufficient to inhibit or kill the growth of microorganisms within the local environment, the incidence of medical device infection will be markedly reduced. There have been reports, albeit limited, of the use of bioactive medical devices for the control of infection. For example, Raad et al (1997) examined the comparative resistances of uncoated catheters and catheters that were coated with minocycline and rifampicin. They reported that 5% of patients developed catheter-related bloodstream infection in the control group whereas there were no cases in the group that had received the coated catheters. More recently, the same group reported a lower incidence of catheter-related bloodstream infection associated with the use of silicone catheters that had been

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impregnated with minocycline and rifampicin in comparison with conventional silicone catheters (Hanna et al 2004). Similarly, reduced microbial colonization of catheters that had been coated with silver sulfadiazine and chlorhexidine has been reported (Brun-Buisson et al 2004; Carrasco et al 2004).

In the design of bioactive biomaterials as medical device coatings, consideration of the physicochemical properties is required as these properties ensure integrity, lubricity and biocompatibility of the coating. Similarly, to achieve optimum resistance to infection the release of the antimicrobial agent from the medical device must be controlled to ensure that sufficient concentration of drug is maintained at the medical device–biological fluid interface for a prolonged period under conditions relevant to the proposed clinical application. However, in spite of the importance of these aspects, there have been few studies that have concurrently examined the effects of physicochemical properties of polymer coatings on the rate and mechanism of drug release. One exception to this is the study by Jones et al (2002b) in which drug release from and the mechanical and microbial anti-adherent properties of hexetidine-incorporated PVC were described. Interestingly, the authors reported the deleterious effects of certain drug loadings on the mechanical properties, thereby highlighting the delicate balance between drug incorporation and the physicochemical properties of biomaterials.

Hydrogels are crosslinked, water-swollen hydrophilic polymers that have been used widely as medical device coatings on catheters and stents and as devices in their own right, e.g. intraocular lenses. It has been reported that hydrogels offer good resistance to microbial adherence (Cormio et al 1996; Tunney et al 1997). Conceivably this resistance may be further enhanced by the controlled release of antimicrobial agents from these polymeric platforms. However, the study of factors that affect drug release from these systems, particularly in light of the nature of the required release profiles for medical device application and the requirement for satisfactory mechanical properties, have not been fully addressed. Therefore, this study examined the effects of crosslinker type and concentration on the physicochemical properties and subsequent release of two model antimicrobial agents from a model cationic hydrogel biomaterial composed of poly(dimethylaminoethylmethacrylate-co-vinylpyrrolidone). This hydrogel has shown potential microbial anti-adherent properties and, therefore, the ability of this biomaterial to control and prolong the release of antimicrobial agents, without detrimentally affecting the physicochemical properties, would be advantageous. In particular, this study will provide an important insight into the relationship between the physicochemical properties of hydrogels and their ability to offer controlled release of antimicrobial agents within a medical device scenario.

Materials and Methods

Materials

2,2′-Azobis-2-methylpropionitrile (AIBN) was purchased from Janssen Chimica (Beerse, Belgium). Dimethylamino ethylmethacrylate, vinylpyrrolidone, tetraethyleneglycol diacrylate (TEGDA), fusidic acid and chlorhexidine were obtained from Sigma Chemical Co (Poole, Dorset, UK). Diethyleneglycol dimethacrylate (DEGDMA) was purchased from Lancaster (Eastgate, UK) whereas polyethylene glycol dimethacrylate (PEGDMA; $n = 200$) was obtained from Aldrich Chemical Company (Dorset, UK).

All other chemicals were AnalaR, or equivalent, grade and purchased from Sigma Chemical. Co. (Poole, Dorset, UK)

Manufacture of drug-containing hydrogels

Copolymers of vinylpyrrolidone and dimethylaminoethylmethacrylate were produced by free radical polymerization in the presence of crosslinkers, as previously described (Jones et al 1997, 2005). In this the appropriate mass of each monomer (1:1) was mixed using a mechanical stirrer and, into this DEGDMA, PEGDMA or triethyleneglycol diacrylate (3, 5 or 10% w/w of monomer) and then AIBN (0.25% w/w) were added and thoroughly mixed until dissolved. This reaction mixture was then added into a cylindrical mould and heated at 80°C for 10 h. Following this, the polymer was stored in deionized water for two days (to ensure removal of unreacted monomers), dried and then immersed in solutions of fusidic acid (1.0% w/w, equivalent to 0.002 mol) or chlorhexidine diacetate (1.5% w/w, equivalent to 0.002 mol chlorhexidine) in deionized water for three days before further analysis.

Examination of drug release

The release of fusidic acid and chlorhexidine from the various biomaterials as a function of time of immersion in selected dissolution media was performed as previously described (Medlicott et al 1996; Jones et al 2000c, 2002b, 2004c). Sections of the various hydrogels were immersed into beakers containing the appropriate dissolution fluid that had been pre-warmed to 37°C and the beakers incubated at 37°C in a shaking water bath ($100\,\text{osc}\,\text{min}^{-1}$). Sink conditions were maintained throughout this period. At predetermined intervals, samples of dissolution fluid (5 mL) were removed and an equal volume of fresh, pre-warmed dissolution fluid replaced into the beakers. The mass of chlorhexidine or fusidic acid in the samples of dissolution fluid were analysed by ultraviolet spectroscopy (λ_{max} chlorhexidine 254 nm, λ_{max} fusidic acid 240 nm) with reference to a previously constructed calibration curve ($r > 0.99$). In total three different dissolution conditions were examined. These were citrate buffer (0.01 M) at pH 6.0, glycine–sodium hydroxide buffer (0.01 M) at pH 9.0, and glycine–sodium hydroxide buffer (0.01 M) containing 0.53% w/w calcium chloride hexahydrate, designed to mimic urine in patients with ureteral stent encrustation.

To determine the mechanism of drug release, the data generated from these release studies were fitted to the general release equation (Peppas 1985; Jones et al 2000c)

using logarithmic transformations and least squares regression analysis, as described below:

$$\log \frac{M_t}{M_\infty} = \log k + n \log t \quad (1)$$

Where M_t is the mass of drug released at time t, M_∞ is the total drug content, k is a constant incorporating structural and geometrical characteristics of the drug delivery system and refers to the fraction of drug released at unit time, and n is the release exponent from which the mechanism of drug release may be elucidated.

In all cases five replicates of each drug release experiment were performed and the validity of the linear relationship was established using an analysis of variance and correlation analysis.

Determination of hydrogel contact angle

The advancing and receding contact angles of the various drug-containing hydrogels were measured using a CAHN Dynamic Contact Angle Analyser (DCA 312). Calibration of the apparatus was performed using a 500 mg tantalum weight. Each (hydrated) sample was immersed into a beaker of reagent grade 1 water at a defined rate (150 μm s^{-1}) to a depth of 10 mm and then removed at the same rate. In all cases five replicates of each analysis were performed. The advancing contact angle was calculated using the software provided. In all cases the advancing contact angles of at least five replicate samples were determined (Jones et al 1997, 2002a, b).

Examination of the mechanical properties of the hydrogels using tensile analysis

The tensile properties of the drug-containing hydrogels were measured using a Stable Micro Systems TA-XT2 Texture Analyser (Godalming, Surrey, UK) as previously described (Gorman et al 1997; Jones et al 2000a, 2002b). In brief, hydrated cylindrical samples (6-cm length $\times$ 0.5-cm diameter) were clamped between the two grips, allowing a defined length of sample between the grips (4 cm). The upper clamp was then raised at a fixed rate of 0.2 mm s^{-1} until fracture of the film occurred. From the resultant relationship between stress and strain the ultimate tensile strength, Young's Modulus and elongation at break (%) were calculated. In all cases five replicates of each analysis were performed.

Equilibrium swelling studies of hydrogels

To determine the swelling properties, the five replicate samples of the hydrogels were dried until constant weight, accurately weighed and then stored in buffer in accordance with a published method (Jones et al 2005). At predetermined times, the samples were removed, excess water removed by blotting with filter paper and reweighed. The swelling ratio was then calculated as the ratio of the water absorbed by the hydrogel to the initial dried weight of the hydrogel.

Modelling of the uptake of buffer was performed using a modification of equation 1:

$$\log \frac{M_t}{M_\infty} = \log k + n \log t \quad (2)$$

Where M_t is the mass of buffer absorbed by the hydrogel following immersion for a period t, M_∞ is the equilibrium buffer uptake, k refers to the fractional uptake of buffer at unit time, and n is an exponent from which the mechanism of buffer uptake may be elucidated.

Evaluation of the validity of this relationship was performed using linear regression analysis in association with an analysis of variance and, additionally by determination of the correlation coefficient.

Statistical analysis

The effect of crosslinker concentration (3, 5, 10%, w/w) and crosslinker type (DEGDMA, PEGDMA or TEGDA) on the mechanical properties (tensile strength, percentage elongation at break and Young's modulus), advancing and receding contact angles, swelling ratio and drug release (mass of drug released at 5 h) were statistically examined using a two-way analysis of variance. Individual differences between the physicochemical and drug release properties of the different crosslinkers and the different concentrations of crosslinkers were statistically analysed using a post hoc test (Tukey's Honestly Significant Difference test). In all cases $P < 0.05$ was accepted to denote significance and accordingly individual probability values were not recorded in the text.

Results

The effects of crosslinker type and concentration on the advancing and receding contact angles of poly(diethylaminoethylmethacrylate-co-vinylpyrrolidone) hydrogels are shown in Table 1. The concentration but not the type of crosslinker affected the contact angles of the hydrogels. In particular, increasing the concentration of crosslinker decreased the advancing contact angle, independent of crosslinker type, but did not significantly affect the receding contact angle. Whilst individual differences

Table 1 The effect of crosslinker type and concentration on the advancing and receding contact angles of poly(diethylaminoethylmethacrylate-co-vinylpyrrolidone) hydrogels

Crosslinker	Crosslinker concn (% w/w)	Contact angle (°) (mean ± s.d.)	
		Advancing	Receding
TEGDA	3	60.37 ± 3.95	45.53 ± 4.50
TEGDA	10	48.00 ± 5.54	45.73 ± 5.54
DEGDMA	3	53.24 ± 5.22	37.21 ± 3.97
DEGDMA	10	43.15 ± 3.11	38.21 ± 3.04
PEGDMA	3	56.87 ± 4.28	41.10 ± 3.57
PEGDMA	10	42.19 ± 3.44	38.22 ± 5.05

Table 2 The effect of crosslinker type and concentration on the tensile and swelling properties of poly(diethylaminoethylmethacrylate-co-vinylpyrrolidone) hydrogels

Crosslinker	Crosslinker concn (% w/w)	Tensile properties (mean ± s.d.)			Swelling ratio (mean ± s.d.)
		Ultimate tensile strength (kPa)	% Elongation at break	Young's modulus (MPa)	
TEGDA	3	35.56 ± 3.58	4.31 ± 1.22	0.82 ± 0.01	3.38 ± 0.09
TEGDA	10	62.17 ± 6.87	1.44 ± 0.20	4.61 ± 0.58	1.91 ± 0.04
DEGDMA	3	27.58 ± 0.98	5.78 ± 0.41	0.38 ± 0.02	4.41 ± 0.12
DEGDMA	10	43.51 ± 2.54	2.64 ± 0.11	1.62 ± 0.09	2.62 ± 0.08
PEGDMA	3	17.55 ± 0.54	5.98 ± 0.61	0.30 ± 0.01	5.84 ± 0.28
PEGDMA	10	25.99 ± 2.04	2.99 ± 0.13	0.95 ± 0.04	3.40 ± 0.17

were observed between the contact angles of the various biomaterials, the hydrogels were hydrophilic in nature.

The swelling properties of the various hydrogels, expressed in terms of the swelling ratio, are presented in Table 2. The nature of the crosslinker significantly affected the swelling properties. In particular, hydrogels manufactured using TEGDA exhibited significantly lower values of the swelling ratio, whereas hydrogels manufactured using PEGDMA exhibited the greatest swelling properties (5.84 ± 0.28). Increasing the concentration of each crosslinker from 3 to 10% significantly decreased the swelling ratio. At the higher concentration of crosslinker, the hydrogels differed in swelling ratios, the lowest value being associated with TEGDA. The swelling ratios of hydrogels that had been crosslinked with PEGDMA and DEGDMA were statistically similar. Application of equation 2 to the swelling data allowed calculation of the swelling exponent for each hydrogel. In all cases the swelling exponent was approximately 0.5, indicative of diffusion-controlled uptake of buffer.

Table 2 additionally displays the effects of crosslinker concentration and type on the mechanical (tensile) properties of the various hydrogels. Wide ranges of mechanical properties were displayed by the various hydrogels, the values of which were statistically dependent on both the concentration and type of crosslinker. Increasing the concentration of each crosslinker from 3 to 10% w/w significantly increased the ultimate tensile strength and Young's modulus but decreased % elongation at break. The maximum and minimum values of ultimate tensile strength and Young's modulus were associated with hydrogels that were manufactured using TEGDA and PEGDMA, respectively. Furthermore, hydrogels prepared using TEGDA exhibited the lowest % elongation at break, whereas the greatest values of this parameter were displayed by hydrogels that had been prepared using either DEGDMA or PEGDMA.

The release of fusidic acid and chlorhexidine from the various hydrogels under examination is presented in Figures 1 and 2, respectively. Furthermore, the mass of each drug released from the hydrogels following immersion in buffer at pH 6 and, in addition, the release parameters that were derived following application of the generalized release equation (eqn 1) (Peppas 1985; Jones et al 2000c) are presented in Table 3. From this it may be observed that the mass of chlorhexidine released after 5 h was greater than that of fusidic acid. Conversely, the kinetic constant associated with chlorhexidine was less than that associated with fusidic acid. For both antimicrobial agents, a greater mass of drug was released from hydrogels after 5 h in which PEGDMA was employed as a crosslinker, whilst the mass released from hydrogels that had been crosslinked with either TEGDA or DEGDMA

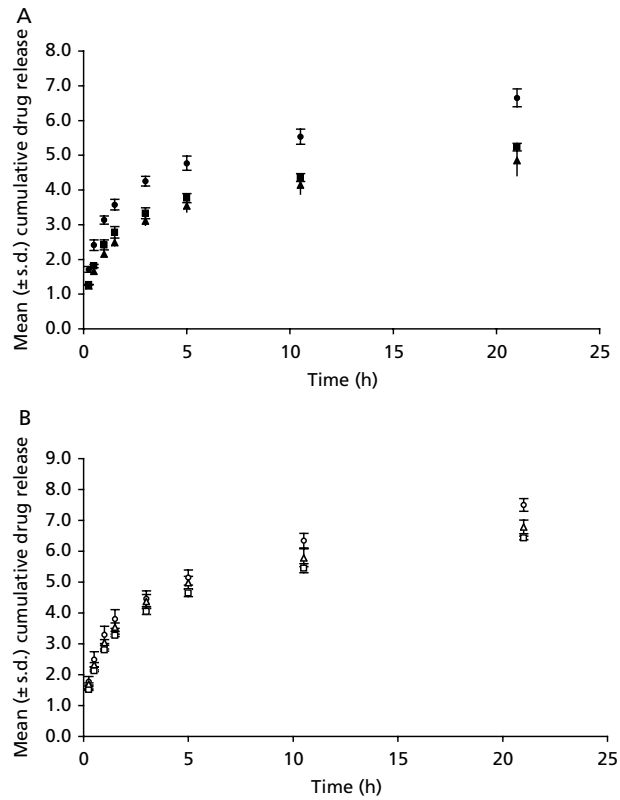

Figure 1 The effect of crosslinker type and concentration on the release of fusidic acid into buffered dissolution medium (0.01 M citrate buffer at pH 6.0). Drug release from hydrogels that had been crosslinked with (A) 3 and (B) 10% w/w crosslinker. Symbols: Circles, squares and triangles refer to drug release from hydrogels crossed with PEGDMA, DEGDMA and TEGDA, respectively.

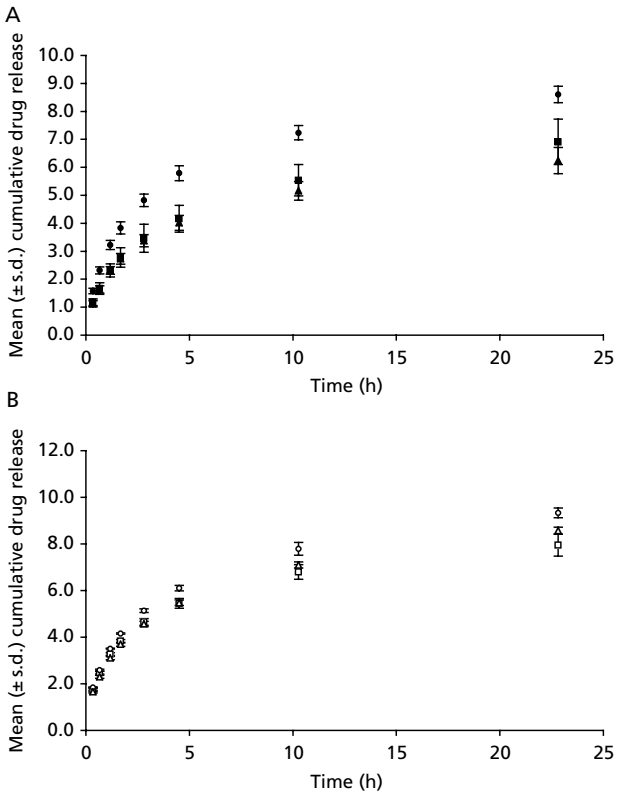

Figure 2 The effect of crosslinker type and concentration on the release of chlorhexidine into buffered dissolution medium (0.01 M citrate buffer at pH 6.0). Drug release from hydrogels that had been crosslinked with (A) 3 and (B) 10% w/w crosslinker. Symbols: Circles, squares and triangles refer to drug release from hydrogels crossed with PEGDMA, DEGDMA and TEGDA, respectively.

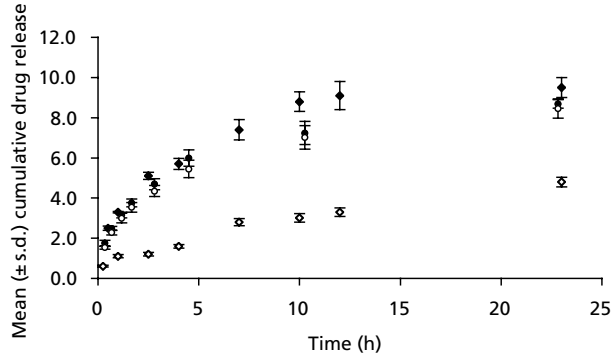

Figure 3 The effect of dissolution medium composition on the release of fusidic acid and chlorhexidine from a poly(DEAEMA-co-VP) hydrogel crosslinked with 3% w/w PEGDMA. Symbols: closed symbols refer to drug release into glycine–sodium hydroxide buffer (0.01 M) at pH 9.0, open symbols refer to drug release into glycine–sodium hydroxide buffer (0.01 M) containing 0.53% w/w calcium chloride hexahydrate (designed to mimic urine in patients with ureteral stent encrustation). Circles and diamonds refer to the release of chlorhexidine and fusidic acid, respectively.

were statistically similar. Similar observations were recorded at other release times. The release exponents associated with the two antimicrobial agents were approximately 0.5. Interestingly, the release exponents and kinetic constants of the hydrogels (containing either fusidic acid or chlorhexidine) were independent of crosslinker type or concentration. However, the kinetic constants associated with hydrogels containing fusidic acid (0.45 ± 0.01) were significantly greater than those observed with chlorhexidine (0.34 ± 0.02). Additional release profiles of chlorhexidine and fusidic acid from poly(dimethylaminoethylmethacrylate-co-vinylpyrrolidone) that had been crosslinked using PEGDMA into release media that had been buffered to pH 9 and either contained or was devoid of supplementary calcium are presented in Figure 3. Notably the inclusion of calcium into the release medium had a pronounced effect on the release of fusidic acid, but not chlorhexidine. In the presence of calcium, the mass of fusidic acid released was decreased at each sampling point in comparison with the release into dissolution fluid devoid of supplementary calcium. Conversely, the presence of calcium in the

Table 3 The effect of crosslinker type and concentration on the release parameters of fusidic acid and chlorhexidine

Crosslinker	Crosslinker concn (% w/w)	Antimicrobial agent	Release parameters[#] (mean ± s.d.)		
			Mass (mg) released after 5 h	Release exponent*	Kinetic constant*
TEGDA	3	Fusidic acid	4.98 ± 0.20	0.41 ± 0.03	0.45 ± 0.02
TEGDA	10	Fusidic acid	3.54 ± 0.17	0.41 ± 0.03	0.44 ± 0.02
DEGDMA	3	Fusidic acid	4.64 ± 0.12	0.42 ± 0.02	0.43 ± 0.01
DEGDMA	10	Fusidic acid	3.76 ± 0.13	0.44 ± 0.02	0.46 ± 0.03
PEGDMA	3	Fusidic acid	5.15 ± 0.25	0.42 ± 0.03	0.44 ± 0.02
PEGDMA	10	Fusidic acid	4.77 ± 0.20	0.41 ± 0.02	0.47 ± 0.03
TEGDA	3	Chlorhexidine	5.48 ± 0.12	0.48 ± 0.02	0.33 ± 0.02
TEGDA	10	Chlorhexidine	4.01 ± 0.27	0.53 ± 0.03	0.33 ± 0.02
DEGDMA	3	Chlorhexidine	5.46 ± 0.21	0.46 ± 0.03	0.37 ± 0.02
DEGDMA	10	Chlorhexidine	4.16 ± 0.48	0.49 ± 0.02	0.32 ± 0.03
PEGDMA	3	Chlorhexidine	6.10 ± 0.11	0.47 ± 0.04	0.35 ± 0.02
PEGDMA	10	Chlorhexidine	5.79 ± 0.26	0.54 ± 0.04	0.33 ± 0.03

[#]Release performed using buffered medium (pH 6).
*Determined using the generalised release equation (see Materials and Methods).

buffered (pH 9) medium did not influence the release of chlorhexidine.

Discussion

Hydrogels may be described as three-dimensional water swollen networks held together by crosslinks formed by covalent, ionic or hydrogen bonds (Anseth et al 1996; Jones et al 2005). The physical properties of hydrogel materials resemble living tissue more so than any other class of synthetic biomaterial, and their resemblance to living soft tissue is shown by their relatively high water content and their soft rubbery consistency (Jones et al 2005). Hydrogels offer a number of advantages compared with other materials used in medical devices and as implantable drug delivery systems (Rosiak & Yoshii 1999). For example, the excellent lubricity of hydrogel-coated catheters ensures ease of insertion into and removal from the urethra thereby minimizing urethral damage (Jones et al 2001a, 2004b). Furthermore there have been reports of the ability of hydrogels to reduce microbial adherence, the initial step in the colonization of medical devices, and hence medical device-related infection (Cormio et al 1996; Tunney et al 1997). As medical device-related infection is frequently associated with morbidity and mortality (Adair et al 1999), this reported ability of hydrogels to reduce microbial adherence is particularly useful.

More recently, it has been accepted that the resistance to medical device-related infection may be further enhanced by the incorporation of antimicrobial agents into the biomaterial or biomaterial coating (Gorman & Jones 2003). In so doing the surface of the biomaterials are rendered antimicrobial and furthermore the subsequent controlled release of the antimicrobial agent into the surrounding biological fluid will result in a microbicidal or microbiostatic effect on the microorganisms that are suspended in this aqueous environment (Jones et al 2002b, 2004a). In light of the ability of hydrogels to resist microbial adherence and the ease of loading of antimicrobial agents into hydrogels by immersion in the appropriate drug-containing solution, this class of biomaterials may be particularly useful for the delivery of antimicrobial agents to biological fluids adjacent to medical devices. Surprisingly there is a paucity of information concerning the role of the physicochemical properties of the biomaterial (hydrogel) coating in the controlled delivery of the antimicrobial agent within the specific demands of bioactive medical devices. Furthermore, in light of the importance of the physicochemical properties to the clinical and non-clinical performance of polymer coatings, it is important to consider these properties in the overall design and development of bioactive biomaterial coatings. Therefore, this study has uniquely concurrently examined the contact angle, mechanical, swelling and drug release properties of a model hydrogel, poly(diethylaminoethylmethacrylate-co-vinylpyrrolidone) that had been crosslinked using a range of crosslinkers. Whilst crosslinkers are frequently employed to modify the mechanical properties of hydrogels, their effects on the surface properties, properties that contribute to the process of microbial adherence, and on the subsequent release of antimicrobial agents for the prevention of medical device-related infection have received limited attention. Therefore, this study aimed to address these deficiencies and to provide insight into the application of hydrogel coatings for the controlled delivery of antimicrobial agents for the prevention of medical device-related infection.

The crosslinkers used in this study, the structures of which are depicted in Figure 4, are typically employed to crosslink acrylate polymers and differ primarily in the number of ethylene oxide repeating units that separate the divinyl components. Accordingly, the distance between adjacent polymer chains will be directly affected by these structural differences, with the greatest distance to be expected in hydrogels that had been crosslinked with PEGDMA. Differences in the mechanical, surface, swelling and drug release properties were observed with different crosslinkers and, additionally, with different concentrations of crosslinkers. The contact angles of all hydrogels under examination were relatively low and therefore the hydrogels may be described as being hydrophilic. Interestingly, the type of crosslinker did not affect the advancing or receding contact angle. Conversely, increasing the concentration of crosslinker from 3 to 10% w/w significantly reduced the advancing but had no effect on the receding contact angle. The effect of increasing crosslinker concentration on the advancing contact angle inferred that a greater proportion of crosslinker was present at the surface of the hydrogel as the concentration of crosslinker was increased. The increased presence of ethylene oxide groups at the surface enhanced the

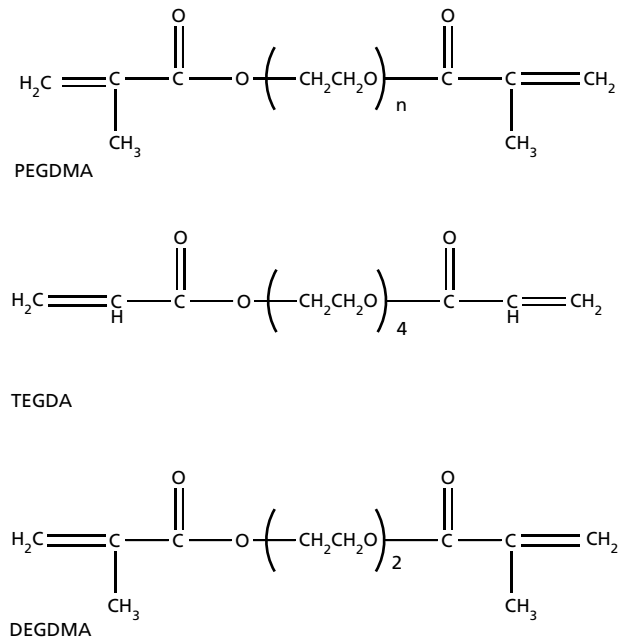

Figure 4 Structures of the crosslinkers used in the study: tetraethyleneglycol diacrylate, TEGDA; diethyleneglycol dimethacrylate, DEGDMA; and polyethyleneglycol dimethacrylate, PEGDMA.

water wettability of the hydrogels. The hydrophilic properties of the three crosslinkers differed (due to the different numbers of repeating ethylene oxide groups). However, these differences were insufficient to affect the advancing contact angles of the various hydrogels.

It is accepted that the mechanical properties of biomaterials, used either as coatings or as devices in their own right, are important properties in their subsequent performance. For example candidate biomaterials should be resistant to deformation without mechanical failure, thereby invalidating the performance of the device. There have been reports of mechanical failure of ureteral stents in-situ, necessitating surgical removal of the fractured pieces (Gorman et al 1997). In the development of bioactive medical device biomaterials it is important to concurrently consider the mechanical and drug release properties. The relationship between these two properties has been reported previously. Jones et al (2000b, 2001a) described the inversely proportional relationship between the (storage) modulus and drug release of tetracycline-containing bioadhesive implants. In this study, the concentration and type of crosslinker directly affected the mechanical properties of the hydrogels. The effects of crosslinker concentration were due to the increased number of polymer crosslinks thereby increasing the ultimate tensile strength, Young's modulus and decreasing the % elongation at break. These observations were in accordance with previous reports (Davis & Huglin 1989; Corkhill et al 1990). The type of crosslinker employed directly affected the mechanical properties; however, no relationship between the molar concentrations of the crosslinker and the observed mechanical properties was observed. Accordingly it may be concluded that the chemical nature of the crosslinker affected the mechanical properties, due to different chemical reactivities leading to differing crosslink densities. In addition, hydrogels prepared using PEGDMA would possess a greater distance between the adjacent polymer backbones and this would accordingly increase the % elongation at break of these polymers. With respect to the suitability of these systems as medical device coatings, the elongations at break of the hydrogels in which the higher concentration of crosslinker was employed were relatively low. This may lead to problems during insertion and flexure of the device in-situ. In this respect, hydrogels prepared using the lower concentration (3%, w/w) of crosslinker would be preferred.

An understanding of the swelling properties of hydrogels is of importance in the design of medical device coatings for two primary reasons. Firstly, the high water content of hydrogels, along with their soft consistency, is in part responsible for the known biocompatibility (St Pourcain et al 1998; Peppas et al 2000). Secondly, the incorporation of drugs within hydrogels is frequently performed by immersion of the dried hydrogel into a solution of the required drug, the mass of drug absorbed being directly proportional to the swelling properties of the hydrogel. Typically the swelling of hydrogels in aqueous solutions is controlled by the compatibility of the polymer with the selected aqueous fluid and by the degree of crosslinking in the hydrogel network (Rosiak & Yoshii 1999; Tritt-Goc et al 2003; Fechner et al 2005). Therefore the effect of crosslinker concentration on the swelling ratio of the hydrogels under examination may be primarily accredited to the increased degree of crosslinking (Davis & Huglin 1989; Corkhill et al 1990). Differences in the effects of the various crosslinkers on the swelling ratio were observed in which hydrogels that were crosslinked with PEGDMA or TEGDA exhibited the highest and lowest degree of swelling, respectively. Again no relationship between the molar concentration of crosslinker and swelling ratio was observed. The hydrophilic nature of the crosslinkers may be ranked according to the number of repeating ethylene oxide units, with DEGDMA and PEGDMA exhibiting the lowest and greatest hydrophilicity, respectively. From the mechanical properties the greatest degree of crosslinking was associated with TEGDA, whereas the PEGDMA-crosslinked hydrogels exhibited the lowest degree of crosslinking. Accordingly, the uptake of buffer was related to the degree of crosslinking and the hydrophilic properties of the crosslinkers. The degree of swelling is an important consideration in the clinical usage of these hydrophilic biomaterials. Ideally, hydrogels should exhibit suitable swelling properties to ensure that a greater mass of drug is incorporated following immersion in the drug-containing solution. In this respect hydrogels prepared with the lower concentration of crosslinker and, in particular, PEGDMA would be suitable.

The release of fusidic acid and chlorhexidine, chosen to represent model acidic and basic antimicrobial agents, from the various hydrogels was observed to be dependent on the crosslinker type and concentration, and on the nature of the release medium. Application of the generalized release equation described by Peppas (1985) allowed an interpretation of the mechanism of drug release from the various hydrogels. Due to the similarity of the release exponents to 0.5, it may be concluded that drug release was diffusion controlled. Therefore, the main determinants of drug release included the active concentration gradient and the diffusion coefficient of the drugs through the polymer matrix. In light of the similarities of the kinetic constants (and hence release rates), it may be concluded that the diffusion coefficient of the antimicrobial agents (chlorhexidine or fusidic acid) was unaffected by the concentration and the type of crosslinker. Due to the aqueous solubility of the chosen antimicrobial agents and the large volume of aqueous fluid, diffusion will occur equally through the aqueous filled channels within each hydrogel matrix, i.e. the aqueous regions that reside between the crosslinked polymer chains. The kinetic constants for each drug were unaffected by the concentration and type of crosslinker. The observed effects of crosslinker type and concentration on the mechanical and swelling properties may thus be accredited, at least in part, to differences in the degree of crosslinking, which would in turn reflect differences in the interchain volume of aqueous fluid. However, as shown by the similarities in kinetic constants, these differences in interchain aqueous fluid volume were insufficient to affect drug diffusion. This was due to the marked differences between the sizes of the diffusing drug molecule and the aqueous regions in the hydrogels. The rate of diffusion of fusidic acid through the

various hydrogels was greater than that of chlorhexidine, which may be accredited to differences in the aqueous diffusion coefficient of the two drugs. Interestingly, the greatest mass of drug released at selected periods was associated with PEGDMA-crosslinked hydrogels whereas, at the corresponding periods, the mass released from hydrogels that had been crosslinked with DEGDMA and TEGDA were similar. As the diffusion coefficient of the drug was unaffected by the concentration and type of crosslinker, this difference may be attributed to the greater mass of absorbed drug within the PEGDMA-crosslinked hydrogels, which, in turn, provided a greater concentration gradient for drug diffusion.

In patients with infection-mediated encrustation the pH of the urine is elevated (to approximately pH 9) due to the bacterial-mediated enzymatic degradation of urea to ammonia and carbon dioxide (Tunney et al 1998). Furthermore, the ionic content of the urine of such patients is different from normal urine, in particular having elevated concentrations of calcium. Therefore, it is important to consider the release of antimicrobial agents from candidate bioactive biomaterials into media that reflects the elevated pH and calcium levels that are typical of this patient group. Accordingly, the release of fusidic acid and chlorhexidine into media buffered to pH 9 and containing an elevated concentration of calcium was described. The concentration of calcium ions was selected according to a previous in-vitro encrustation model that had been described by the authors (Tunney et al 1996a, b). This study has uniquely illustrated the effects of the inclusion of calcium on the diffusion of fusidic acid but not chlorhexidine. In particular the diffusion of fusidic acid was reduced in the calcium-modified medium, which, in light of the acidic properties of this antimicrobial agent, may be accredited to the reduced solubility (and reduced concentration gradient within the hydrogel matrix) of the calcium salt of fusidic acid. Chlorhexidine is a strong base and therefore salt formation with calcium will not occur. Consequently, the diffusion of chlorhexidine was unaffected by the presence of this ion. These observations have direct clinical consequences and illustrate the potential concerns associated with the use of acidic antimicrobial agents in urological devices. It is important to note that the elevated calcium content of the dissolution medium did not inhibit the release of fusidic acid but markedly reduced this parameter. Therefore, in the design of such systems, it is important to characterize drug release in urologically relevant medium as this will allow a more accurate representation of the possible in-vivo release.

In conclusion, this study has examined the effects of crosslinker concentration and type on the physicochemical and drug (fusidic acid and chlorhexidine) release properties of a model hydrogel, which has shown initial promise as a urological biomaterial. Importantly, crosslinker type and concentration directly affected the mechanical and surface properties, water uptake and drug release properties. Based on these properties PEGDMA (3% w/w) showed initial promise as a crosslinker for the hydrogel under examination. Interestingly, the release of fusidic agent (but not chlorhexidine) was affected whenever artificial urine (containing elevated concentrations of calcium) was employed as the dissolution medium; an observation that was accredited to the reduction in the solubility of fusidic acid in the hydrogel matrix and release medium due to the formation of the calcium salt of this antimicrobial agent. Therefore, it is proposed that the in-vitro characterization of the release of acidic drugs from urological biomaterials should utilize a calcium-enhanced dissolution medium. Furthermore, caution should be exercised when using acidic antimicrobial agents in urological biomaterials due to the possible ionic effects on drug release.

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