Novel Porphyrin-Incorporated Hydrogels for Photoactive Intraocular Lens Biomaterials

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Novel surface-modified hydrogel materials have been prepared by binding charged porphyrins TMPyP (tetrakis-(4-*N*-methylpyridyl)porphyrin) and TPPS (tetrakis(4-sulfonatophenyl)porphyrin) to copolymers of HEMA (2-hydroxyethyl methacrylate) with either MAA (methacrylic acid) or DEAEMA (2-(diethylamino)ethyl methacrylate). The charged hydrogels display strong electrostatic interactions with the appropriate cationic or anionic porphyrins to give materials which are intended to be used to generate cytotoxic singlet oxygen ($^1O_2$) on photoexcitation and can therefore be used to reduce postoperative infection of the intraocular hydrogel-based replacement lenses that are used in cataract surgery. The UV/vis spectra of TMPyP in MAA:HEMA copolymers showed a small shift in the Soret band and a change from single exponential (161 μs) triplet decay lifetime in solution to a decay that could be fitted to a biexponential fit with two approximately equal components with $\tau = 350$ and 1300 μs. O_2 bubbling reduced the decay to a dominant (90%) component with a much reduced lifetime of 3 μs and a minor, longer lived (20 μs) component. With D_2O solvent the $^1O_2$ lifetime was measured by 1270 nm fluorescence as 35 μs in MAA:HEMA, compared to 67 μs in solution, although absorbance-matched samples showed similar yield of $^1O_2$ in the polymers and in aqueous solution. In contrast to the minor perturbation in photophysical properties caused by binding TMPyP to MAA:HEMA, TPPS binding to DEAEMA:HEMA copolymers profoundly changed the $^1O_2$ generating ability of the TPPS. In N_2-bubbled samples, the polymer-bound TPPS behaved in a similar manner to TMPyP in its copolymer host; however, O_2 bubbling had only a very small effect on the triplet lifetime and no $^1O_2$ generation could be detected. The difference in behavior may be linked to differences in binding in the two systems. With TMPyP in MAA:HEMA, confocal fluorescence microscopy showed significant penetration of the porphyrin into the core of the polymer film samples (>150 μm). However, for TPPS in DEAEMA:HEMA copolymers, although the porphyrin bound much more readily to the polymer, it remained localized in the first 20 μm, even in heavily loaded samples. It is possible that the resulting high concentration of TPPS may have cross-linked the hydrogels to such an extent that it significantly reduced the solubility and/or diffusion rate of oxygen into the doped polymers. This effect is significant since it demonstrates that even simple electrostatic binding of charged porphyrins to hydrogels can have an unexpectedly large effect on the properties of the system as a whole. In this case it makes the apparently promising TPPS/DEAEMA:HEMA system a poor candidate for clinical application as a postoperative antibacterial treatment for intraocular lenses while the apparently equivalent cationic system TMPyP/MAA:HEMA displays all the required properties.

Introduction

Porphyrins and other tetrapyrroles are used widely in clinical settings as sensitizers for singlet oxygen ($^1O_2$) generation in photodynamic therapy (PDT). PDT has previously been applied successfully to the treatment of tumors, atherosclerotic lesions, and eye conditions.[1] It has also been successfully used in antimicrobial and antiviral applications.[2] For solid tumors, the patient is intravenously injected with a porphyrin "sensitizer" that, after several days, has accumulated within the tumor mass and can be activated by illumination with red light.[3] In antimicrobial and antiviral applications the porphyrin is used in solution.

This paper is concerned with the photophysical characterization of new types of hydrogel materials which have been surface modified by doping with porphyrins to give them photodynamic activity. The objective is to develop new approaches to surface modification of the hydrogel-based intraocular lenses (IOLs) which are implanted during cataract surgery. Post operative infection of these IOLs (infectious endophthalmitis) is a common complication of cataract surgery, which requires prolonged hospitalisation and further surgery.[4] Microorganisms may enter the eye by means of surgical instruments, irrigation fluid, or contamination of the IOL implant itself. Bacterial adherence to a surface is the first step in the infection process and the colonizing bacteria then proceed to encase themselves in a protective glycocalyx composed of exopolysaccharides to form a biofilm with greatly enhanced resistance to removal from the IOL surface and to the eliminating effects of antibiotic therapy.[4]

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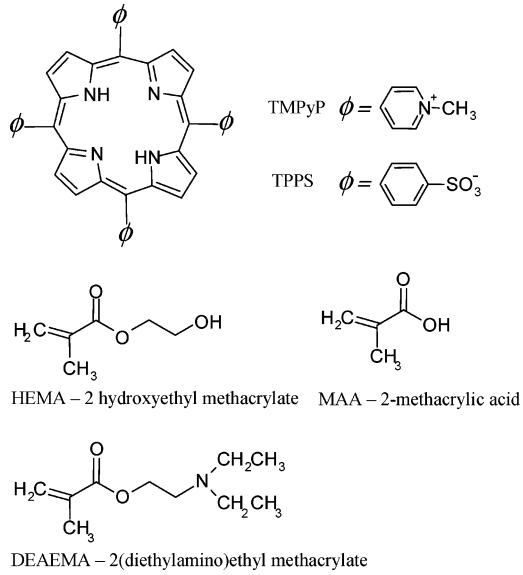

Figure 1. The structures of the porphyrins and monomers used in this study.

In the surface-modified materials discussed here the aim is to concentrate the photocytotoxic effect of the porphyrins at the point where it is required to prevent this bacterial colonization of the material by attaching the porphyrins at (or near to) the hydrogel surface where they will remain inactive until treated with light. On light activation, it is hoped that the porphyrin will produce mobile, highly reactive $^1O_2$, which is known to kill pathogenic bacteria. The lifetime of singlet oxygen is short, $10^{-5}-10^{-6}$ s, which should limit the effective distance between the initial excitation event and the cytotoxic damage to a maximum of a few micrometers. This offers a distinct advantage since the treatment area is strongly localized at the required point, i.e., at the device surface, and obviates the problems associated with incidental toxicity arising from accumulation of sensitizer in normal tissue.

Previous work on porphyrin localization within polymer hosts has predominantly been concerned with development of oxygen sensors based on the quenching of luminescence, typically from Pt(II) porphyrins.[5] The hosts range from silica-based sol gels[6] and polystyrene[7] to perfluorinated ion exchange membranes (e.g., Nafion).[8] Porphyrins located in a sol−gel matrix have also been used for pH sensing.[9] Use of porphyrin loaded polymers in PDT has been confined to either development of nanomaterials for delivery[10] or systems aimed, ultimately, at water purification.[11]

In this work hydrogels were used as the polymer host because of the widespread use of hydrogels in IOL manufacture. Two general types of model hydrogel were investigated, these were copolymers of HEMA (2-hydroxyethyl methacrylate) with either MAA (methacrylic acid) or DEAEMA (2-(diethylamino)ethyl methacrylate), to provide anionic or cationic pendant groups, respectively. These charged hydrogels are expected to have strong electrostatic interactions with charged porphyrins and in this work TMPyP (tetrakis(4-*N*-methylpyridyl)porphyrin) and TPPS (tetrakis(4-sulfonatophenyl)porphyrin) were used as generic cationic and anionic porphyrins. The structures of the monomers and porphyrins are shown in Figure 1. A range of copolymer compositions was investigated to determine if inclusion of larger proportions of the charged monomers had a significant effect on the porphyrin binding. This paper deals with the binding, photophysics, and release of the porphyrins in these polymer hosts; the results of microbiological testing will be reported separately.

Materials and Methods

Sample Preparation. Copolymers were produced by free radical polymerization in the presence of cross-linkers, as previously described.[12] Copolymers composed of a variety of compositions of HEMA and MAA were prepared by mixing the required amounts of HEMA, MAA, cross-linking agent (EGDMA, ethylene glycol dimethacrylate, 1% w/w), and the initiator (benzoyl peroxide (BPO), 0.4% w/w) in a flat-bottomed beaker. The mixture was mechanically stirred until the benzoyl peroxide had fully dissolved. The solution was then injected into a mould, made from medical grade tubing placed between two sides of silicone release liner held together by two glass plates. The plate moulds were then placed in a fan-assisted oven maintained at 90 °C for 2 h, during which time the polymerization reaction occurred. This procedure generated flat sheets which were at least 100 mm × 100 mm and whose thickness was controlled by the plate spacing (typically ca. 0.75 mm). The cationic copolymers of DEAEMA and HEMA were synthesized by using a similar method; AIBN (2,2-azobis(2-methylpropionitrile) (1% w/w)) was used instead of BPO as the initiator and the polymerization was in an oven maintained at 60 °C for 18 h. On removal from the moulds, the films were washed with deionized water, cut into samples of a convenient size (ca. 10 × 20 mm), and then immersed in deionized water for 14 days to remove any unreacted monomer before use.

The porphyrins were loaded into the polymer films by immersing the 10 × 20 mm film samples for 60 s into high concentration (1−100 μg/mL, depending on loading level required) solutions of the complementary porphyrin (TMPyP for MAA:HEMA, TPPS for DEAEMA:HEMA). Fine control of the loading was achieved by repeating the immersion process. No significant differences were noted between repeated immersions and a single loading step provided it corresponded to the same total immersion time; the repeated immersion method was used solely because it allowed more control of the doping level in the final product. The polymer films were presoaked in Tris (tris(hydroxymethyl)aminoethane) buffer before being treated, since this circumvented any potential complications which might be associated with loading porphyrin into dry polymer samples where solvent ingress and polymer swelling would necessarily occur in parallel with incorporation of the sensitizer.

Instrumental Techniques. Electronic absorption spectra were recorded on a Hewlett-Packard HP8453 diode array single beam spectrophotometer with 2 nm resolution over a 190−820 nm wavelength range. Fluorescence spectra were measured with a Perkin-Elmer LS55 luminescence spectrometer equipped with a R928 photomultiplier.

For the transient absorption and singlet oxygen studies, the second harmonic output from a Q-switched Nd:YAG laser was used as the excitation source and the sample was mounted at 45° to the excitation beam. A pulse energy of 1 mJ was used for the measurements to avoid burning the sample. For transient absorption measurements, light from a xenon arc lamp (Applied Photophysics Ltd., 150 W) travelling at 90° to the excitation beam was directed through the sample and into a monochromator (Applied Photophysics Ltd., 1200 g/mm grating) fitted with an IP28 photomultiplier detector connected to a sampling oscilloscope (Tektronix TDS 3032). A 532 nm holographic notch filter (Kaiser Optical Systems Inc.) was mounted in front of the monoochromator entrance slit to reduce the effect of laser

scatter on the signal. Processing of the data was carried out with SigmaPlot for Windows (Version 8.0).

The $^1O_2$ detection system was a liquid-nitrogen-cooled Indium Gallium Arsenide (InGaAs) detector (Judson Technologies Inc., Montgomeryville, PA, type J22D-M204-R01M-60-1.7) with a 1 mm^2 active area. The detector output was amplified with a Judson PA9 preamplifier and collected with a Tektronix TDS 3032 oscilloscope. To obtain acceptable signal-to-noise ratios 512 decays were summed for each reading. To minimize the effect of any inhomogeneity in the sample, readings from 8 different points were averaged. The singlet oxygen emission at 1270 nm was separated from the 1064 nm fundamental laser emission and other spurious emissions by use of a 1200 nm long pass filter (LP1200) and a 1292 nm band-pass filter (BPO-1292-80), both supplied by Spectrogon UK Ltd.

Confocal laser scanning microscopic (CLSM) examination of samples was carried out with a Leica TCS SP2 confocal laser scanning microscope. After focusing, the sample surface was excited by using the 514 nm line from a Ar/ArKr laser and fluorescence emission data collected over the range 600−720 nm. Fluorescence emission micrographs which showed summed photomultiplier intensities across the full wavelength range detected were recorded but are not shown here since displaying the data as intensity versus depth into the sectioned film is more appropriate for measuring penetration depths.

Contact angles of the copolymers were measured with a First Ten Angströms FTA 200 video-based contact angle analyzer. All measurements were carried out at room temperature on hydrated materials in a three-phase system consisting of deionized water/buffered solution, the surface of the material, and a bubble of air. The sample was placed on top of two inert plastic supports in the liquid chamber and a bent needle syringe shaped in the form of a "J" was used to dispense air bubbles with a volume of 20 μL. These adhered to the lower surface of the sample and were recorded and measured by using the instrument's internal video capture system and software. The contact angle between the air and the sample surface, θ_{air}, was measured for 10 bubbles and the mean value of the complementary angle, θ_{buffer}, was calculated.

Results and Discussion

The objective of this work was to prepare polymer matrices which incorporated charged porphyrins at their surfaces and would thus be capable of generating $^1O_2$ at the point of potential bacterial attachment. Although it is possible to link porphyrins to the surfaces of polymers through covalent bonds[13] we have chosen the much more convenient route of electrostatic binding, in this case simply dipping a small sample of a polymer film into a highly concentrated solution of the complementary porphyrin (TMPyP for MAA:HEMA, TPPS for DEAEMA:HEMA). Our initial tests showed that immersion for even a few seconds resulted in binding of porphyrin to the polymer, which gave it a distinct yellow/orange tint that could not be removed even by vigorous washing. The systematic studies outlined below were aimed at finding which factors are important in determining the properties of copolymer films whose surfaces have been modified by this simple route. Many of the features of the sample preparation and characterization were similar for both anionic and cationic porphyrin/copolymer combinations so for brevity the preparation and characterization of cationic TMPyP in the MAA:HEMA system are described in detail here while for the TPPS/DEAEMA:HEMA system only features which were significantly different from the cationic analogue are discussed.

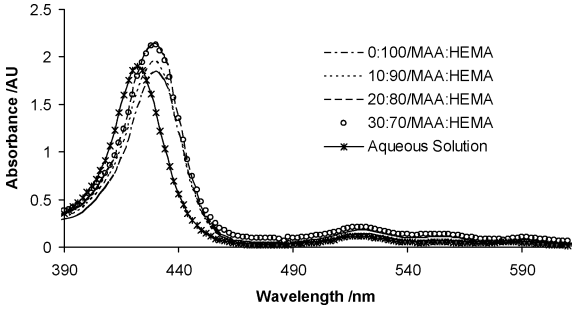

Figure 2. UV/vis absorption spectra of TMPyP in MAA:HEMA copolymers with the compositions marked on the figure. Samples were prepared by dipping 5 times in 100 μg/mL porphyrin solution. The spectrum of a simple aqueous solution of the porphyrin has been included for comparison.

Loading of Porphyrins into Copolymer Films. The UV/vis spectra of all the porphyrin-loaded films were dominated by the porphyrin content and provided a useful method for determining total porphyrin loading (in μg cm^{-2}; measurement of local concentration, which varied with distance from the surface, is discussed below). Initial experiments with the MAA:HEMA copolymers were carried out on samples between 0:100/MAA:HEMA and 100:0/MAA:HEMA but it was found that the samples with >30% MAA were cloudy. Subsequent studies were confined to the transparent polymers with 0%, 10%, 20%, and 30% MAA. In part, this was because the optical characterization was complicated by light scattering effects but, more importantly, since the ultimate application of the materials is in lens materials, samples with poor optical qualities would be unsuitable in any case.

It was necessary to prepare samples with very different porphyrin loadings by making gross concentration changes to the porphyrin solution because some of the characterization methods required much higher loadings than others. For example, flash photolysis measurements could be carried out on MAA:HEMA samples which were dipped 5 times into 10 μg/mL solutions of TMPyP. These low loading measurements were useful because treatments that give strongly colored polymers will not be acceptable for clinical use and at these loading levels the samples had only a faint yellow tint. However, higher loadings were needed for singlet oxygen measurements, so for these experiments the samples were prepared in the same way but 100 μg/mL solutions were used. This method gave films with a peak absorbance ca. 1−2 at λ_{max}, which were also suitable for UV/vis absorption measurements.

It is well-known that λ_{max} of the Soret band of tetraaryl porphyrins shifts in different chemical environments, for example λ_{max} of H_2TPPS shifts 2 nm on incorporation in cationic functionalized polystyrene beads[11] and TMPyP shows a 22 nm bathochromic shift (422−444 nm) on binding to poly(dG-dC).[14] It can be difficult to associate these shifts to particular binding motifs since they may be associated with nonspecific effects, such as a modification of the polarity of the medium, or they may arise from weak electronic interactions between the porphyrin substituents and binding groups on the host. In addition, the formation of dimers/oligomers in the polymer film or dissociation of aggregates which were present in solution but not in the films could also give significant shifts in the porphyrin absorption.[15] Figure 2 shows that λ_{max} for TMPyP in MAA:HEMA shifts slightly to the red when it is bound to MAA:HEMA. (430 nm for bound porphyrin, 424 nm for TMPyP in aqueous solution). The shift was found to be essentially identical for all the polymer compositions implying that the environment around the bound porphyrins was similar at

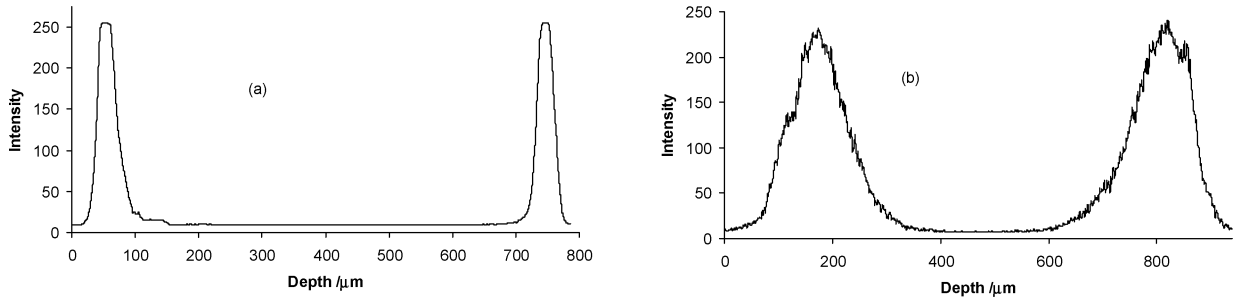

Figure 3. Depth profiles of the fluorescence intensity of TMPyP in 20:80/MAA:HEMA films measured by confocal laser fluorescence microscopy. Samples were prepared by immersing the polymer sample either (a) once or (b) 5 times in 100 μg/mL porphyrin solution.

all compositions. Similarly, the uptake, as determined from the absorbance at λ_{max}, was also very similar for each of the polymers (see Figure 2). A slightly higher absorbance was observed for the 30% and 20% MAA than the 10% but, somewhat surprisingly, the extent of incorporation into the 100% HEMA was almost as high as that in the MAA:HEMA copolymers, despite the fact that that no anionic groups were deliberately introduced into the polymer. It is possible that incomplete esterification of the HEMA resulted in a small population of residual uncapped anionic binding sites even in "100%" HEMA samples, which is consistent with the similar shift in λ_{max} observed in all the polymers studied. The release studies discussed below do, however, suggest that the TMPyP was less strongly held in 100% HEMA than in the MAA:HEMA polymers.

Since the samples were prepared by immersion of films into porphyrin solutions, initial incorporation must necessarily have been into surface layers but it was not obvious whether the porphyrins would penetrate the films to a significant depth or would remain in a high concentration layer at the surface. Confocal laser fluorescence microscopy of MAA:HEMA films showed that after 1 immersion cycle the porphyrin was indeed localized in a <50 μm surface layer and there was no evidence for significant penetration of porphyrin from the surface to the core (Figure 3a). Repeating the immersion cycle 5 times gave samples with increasing loading levels whose depth profiles showed evidence of an outer layer >100 μm, which appeared to be saturated with porphyrin, and deeper into the film the concentration decreased nonlinearly over the next $50-100$ μm (see Figure 3b). This profile is at least consistent with a model of the loading process where porphyrins diffuse from the exterior surface into the bulk material but diffusion stops once they encounter a suitable binding site. In this model the first porphyrins to enter the polymer will have a high probability of encountering binding sites near the surface and become localized there. This will continue until all the binding sites near the surface are occupied (i.e., a layer of polymer saturated with porphyrin will form at the surface). Subsequent porphyrins will then be forced to diffuse through the saturated layer to find vacant binding sites and since the probability that they will bind near the saturated region will be higher than the probability for further diffusion into the bulk the saturated region will advance inward, although some porphyrin may randomly penetrate more deeply, giving rise to a concentration of porphyrin that falls off with distance from the saturated layer. Release studies (see below) imply that it is difficult for any bound TMPyP to release, which rules out the alternative model where porphyrins penetrate the polymer through a series of binding/release steps.

The fluorescence profiles also allowed the porphyrin concentration within the films to be estimated. UV/vis absorption spectroscopy (Figure 2) gave the loading per cm^2 of film but this is a global value that does not take account of the inhomogeneous depth profile. However, the confocal fluorescence data (Figure 3) for the 20:80/MAA:HEMA polymer, for example, show that the porphyrin can be regarded as confined to 2 layers ca. 180 μm thick which to a first approximation are uniformly loaded with TMPyP. Taken with the peak absorbance and assuming an extinction coefficient of 2.26×10^5 dm^3 mol^{-1} cm^{-1} gives a concentration in these surface layers of 2×10^{-4} mol dm^{-3}. For all the MAA:HEMA compositions studied the extent of penetration by the porphyrin was found to be similar when similar loading conditions were used.

TABLE 1: Contact Angles for MAA:HEMA Copolymers of Varying Composition Treated with TMPyP

	θ/deg		
MAA:HEMA composition	blank	100 μg/mL of TMPyP	1 μg/mL of TMPyP
30:70	34.4 ± 2.5	23.8 ± 3.5	23.7 ± 0.6
20:80	41.6 ± 1.2	22.7 ± 1.4	24.1 ± 1.8
10:90	47.0 ± 4.3	24.5 ± 1.7	23.7 ± 3.1
0:100	48.3 ± 2.1	24.7 ± 2.8	25.8 ± 1.6

Contact angle measurements (Table 1) clearly show that the surface properties are established even at low TMPyP loading since the wide range of θ values observed for the blank polymers converge to a single value of ca. 24° on first treatment and do not alter as the porphyrin loading is increased. It is interesting that, despite the significant variation in contact angle for the untreated polymers with different compositions, the treated samples all have the same contact angle. This suggests that the surface properties of the modified polymers are dominated entirely by the porphyrins, which can change the contact angle by up to 22°.

The general features of TPPS incorporation into DEAEMA:HEMA copolymers were similar to those of the TMPyP system described above. λ_{max} of the polymer bound TPPS also showed a similar bathochromic shift compared to solution ($\lambda_{max} = 412$ nm in solution, 420 nm in 10:90/DEAEMA:HEMA) but, as shown in Figure 4, the 40:60/DEAEMA:HEMA showed clear evidence of two different types of binding with a shoulder on the Soret band at 410 nm, near the position of the solution λ_{max}. The occurrence of different binding domains within copolymers of this type is not unexpected.

One significant difference between the TPPS and TMPyP systems was the extent of sensitizer uptake for a given concentration of loading solution. With MAA:HEMA, dipping the films 5 times into 100 μg/mL solutions of TMPyP gave samples with absorbance ca. 2 at λ_{max}; with DEAEMA:HEMA loading with 100 μg/mL TPPS solutions gave very heavily doped dark red samples and much lower concentration (10 μg/mL) TPPS solutions were required to generate samples with an appropriate absorbance at λ_{max}. Confocal laser scanning microscopy also showed a significant difference in the distribution of porphyrin in the anionic and cationic polymer systems.

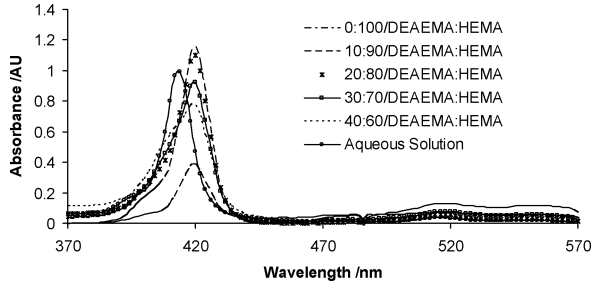

Figure 4. UV/vis absorption spectra of TPPS in DEAEMA:HEMA copolymers with the compositions marked on the figure. Samples were prepared by dipping 5 times in 10 μg/mL porphyrin solution. In the 10%, 20%, and 30% DEAEMA samples the spectra show a simple bathochromic shift compared to solution ($\lambda_{max} = 412$ nm in solution, 420 nm in 10:90/DEAEMA:HEMA). However, 40:60/DEAEMA:HEMA shows clear evidence of two different types of binding with a shoulder on the Soret band at 410 nm, near the position of the solution λ_{max}.

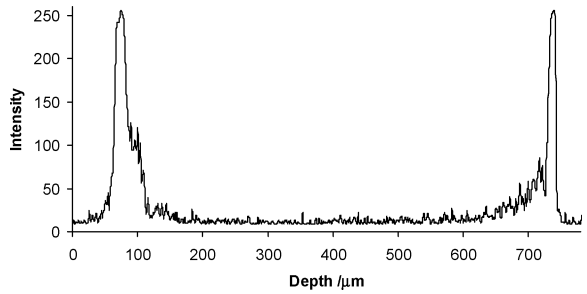

Figure 5. Depth profiles of the fluorescence intensity of a TPPS 30:70/DEAEMA:HEMA film measured by confocal laser fluorescence microscopy. Samples were prepared by immersing the polymer sample in 100 μg/mL porphyrin solution. There is remarkably little penetration of the TPPS into the body of the copolymer film.

TABLE 2: Contact Angles for DEAMA:HEMA Copolymers of Varying Composition Treated with TPPS

DEAEMA:HEMA composition	θ/deg	
	blank	100 μg/mL of TPPS
30:70	32.2 ± 0.8	27.3 ± 1.3
20:80	36.4 ± 1.6	29.7 ± 2.8
10:90	41.3 ± 3.2	29.6 ± 3.7
0:100	48.0 ± 2.4	31.8 ± 0.2

In contrast to the MAA:HEMA polymers, where the TMPyP formed a band >150 μm deep, the 30:70/DEAEMA:HEMA showed remarkably little penetration of the TPPS into the body of the copolymer film, even after 5 immersions the porphyrin was confined to a narrow band (fwhm <20 μm) at the film's surface (see Figure 5). Presumably, in this case the initial binding of the charged polymers restricts the ingress of additional porphyrin so the adsorbed material remains concentrated near the surface (local porphyrin concentration is estimated as ca. 4 × 10^{-3} mol dm^{-3}, i.e., >20× larger than TMPyP in the MAA:HEMA polymers). Contact angle measurements (Table 2) show that binding the porphyrin to the surface does significantly alter the contact angle by up to 16° and that binding TPPS resulted in a near identical contact angle for all the polymer compositions investigated, irrespective of their untreated values. However, this behavior cannot be directly linked to an unusually strong surface binding by TPPS because similar effects were observed for TMPyP in the MAA:HEMA system where the porphyrin penetrated much more deeply into the copolymer film.

Photophysical Studies. Transient absorbance difference (ΔA) measurements were carried out under oxygen-bubbled conditions and after degassing by nitrogen bubbling for 20 min. Although N_2 bubbling is much less effective at removing oxygen than repeated freeze−pump−thaw cycles it was used here because freeze−pump−thaw was inappropriate for the polymer samples. The gross photophysical properties of TMPyP in MAA:HEMA polymers were similar to those of simple aqueous solution, the triplet had a ca. 1 ms lifetime in deoxygenated polymer which fell to ca. 3 μs under O_2 saturation. The solution-phase values we have measured are 161 μs under N_2 bubbling, falling to 436 ns under O_2.[12]

The solution-phase data were always pure simple exponential decays within experimental error but the residuals from single-exponential fits to the decay curves of the polymer samples showed that the traces were not single exponential. This is not surprising since the polymers are microheterogeneous and the porphyrins may therefore be located in a broad range of environments. Under N_2-bubbled conditions the triplet signals could be fitted to two approximately equal intensity components with lifetimes ca. 350 and 1300 μs (see Table 3) although this was an approximation at best and the residuals to this biexponential fit showed some structure, despite the $R^2 = 0.99$ (Figure 6). It is likely that numerous different environments exist within a single polymer sample and the values of the fit give only an approximate indication of the range of lifetimes present and the relative proportions of longer and shorter lived components. Similarly, in the oxygen-bubbled polymers the lifetimes were not single exponential, although in this case there was a single dominant shorter component (ca. 90% of the signal) with $\tau =$ 3 μs and a minor longer lived component with a significantly longer lifetime of 20 μs. Presumably this longer lived component arose from porphyrins which lay within domains where O_2 has low solubility and/or diffusion rates. Interestingly, there was no evidence of a systematic change in the relative contribution of these two components with changing polymer composition.

The strong quenching of triplet TMPyP in the polymers by molecular oxygen shows that even the porphyrins that lie deep within the polymers are exposed to O_2 (all lifetimes are reduced by >1 order of magnitude on O_2 bubbling). For the purposes of antimicrobial activity it is only the surface that is important but the fact that underlying porphyrins were also quenched was useful here because it meant that heavily doped samples gave more $^1O_2$ than samples with low loadings and thus gave detectable signals.

In initial experiments with an InGaAs detector for direct detection of $^1O_2$ fluorescence at 1270 nm it was found that TMPyP loaded MAA:HEMA copolymers give a strong emission signal at 1270 nm even under degassed conditions. This emission showed a bandwidth-limited rise and decay (2.5 and 3.5 μs, respectively) and did not appear to be due to an impurity in the polymer since it was also observed for TMPyP in simple aqueous solution, while experiments on undoped polymer showed no signal. A similar fast emission signal at 1270 nm has previously been observed for TPPS dissolved in H_2O.[16] Although the origin of the emission signal is unclear it was previously found that if D_2O was used as the solvent the increased $^1O_2$ lifetime (62 μs vs. 3 μs in H_2O) allowed the $^1O_2$ signal to be separated from the much shorter emission signal. Here the same approach was used, the polymers were prewetted in buffer prepared with D_2O and dipped in porphyrin solutions also prepared from D_2O. Figure 7 shows $^1O_2$ emission from 4 doped polymer samples and from a solution that was absorbance matched. The solution phase, 0:100/MAA:HEMA and 10:90/MAA:HEMA samples show fluorescence yields (measured after the fast decaying initial emission) identical within the ca. 20%

TABLE 3: Transient Absorbance Lifetime Data for Porphyrin-Treated Copolymer Samples and Simple Aqueous Solutions of The Same Porphyrins[a]

material composition	conditions	τ_1/μs (%)	τ_2/μs (%)
MAA:HEMA			
30:70	N_2 bubbled	230.1 ± 71.8 (47)	1414.5 ± 142.0 (53)
	O_2 bubbled	2.9 ± 0.04 (87)	23.7 ± 0.7 (13)
20:80	N_2 bubbled	289.8 ± 104.5 (58)	1113.6 ± 165.2 (42)
	O_2 bubbled	2.8 ± 0.1 (90)	26.2 ± 4.1 (10)
10:90	N_2 bubbled	487.5 ± 76.7 (70)	1388.3 ± 228.8 (30)
	O_2 bubbled	2.8 ± 0.1 (92)	37.9 ± 1.2 (8)
0:100	N_2 bubbled	1097.8 ± 4.1	
	O_2 bubbled	3.5 ± 0.005 (94)	13.5 ± 0.07 (6)
solution	N_2 bubbled	161.0 ± 4.51	
	O_2 bubbled	0.436 ± 0.00062	
DEAEMA:HEMA			
30:70	N_2 bubbled	378.1 ± 2.6 (47)	1182.4 ± 34.2 (53)
	O_2 bubbled	310.2 ± 8.4 (46)	1163.4 ± 46.7 (54)
20:80	N_2 bubbled	471.3 ± 8.3 (33)	1370.0 ± 10.1 (67)
	O_2 bubbled	383.5 ± 10.6 (30)	1127.6 ± 62.7 (70)
10:90	N_2 bubbled	497.0 ± 9.2 (51)	1157.6 ± 38.7 (49)
	O_2 bubbled	302.5 ± 60.4 (31)	1100.4 ± 88.7 (69)
0:100	N_2 bubbled	891.5 ± 6.7 (95)	2280.9 ± 247.65 (5)
	O_2 bubbled	3.22 ± 0.02 (92)	14.34 ± 3.07 (8)
solution	N_2 bubbled	227.5 ± 6.1	
	O_2 bubbled	0.760 ± 0.0023	

[a] Solution data were fitted by a single-exponential decay, polymer samples were fitted by two-component decays with lifetimes and percent contribution to the total signal as shown.

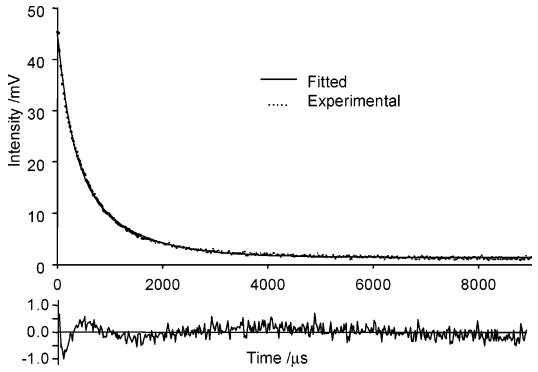

Figure 6. Transient absorbance difference signal (λ_{ex} = 532 nm, λ_{mon} = 474 nm) showing decay of excited TMPyP in 20:80/MAA:HEMA under N_2-bubbled conditions. The upper plot compares experimental data with a fitted biexponential decay. The lower plot shows the residuals to this biexponential fit.

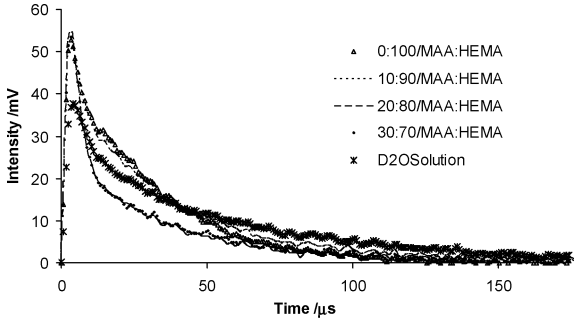

Figure 7. Decay traces for $^1O_2$ (1270 nm) emission from four TMPyP-treated MAA:HEMA copolymers of composition shown on the figure. Data from an absorbance-matched solution are also shown.

experimental uncertainty of the measurements. Following the rapid component, the traces show single-exponential decay with lifetimes of 62 (solution) and 35 μs (polymer). The shorter lifetime in the polymer shows that the $^1O_2$ is not generated and retained within simple solvent pockets in the polymer but instead it must be perturbed by interaction with the host. Similar results would have been expected for the higher MAA content polymer samples but it was found that the intensity of the $^1O_2$ signal following the initial fast transient was only ca. 50% that of the other polymer samples, although the initial signal heights were the same with all the polymers studied. These observations are consistent with an additional fast decay channel being present in the high MAA polymers. However, there is insufficient evidence to advance this as any more than a tentative suggestion.

Overall, the data for TMPyP in MAA:HEMA suggest that loading water-soluble porphyrins into swellable acrylate-based gels leads to only minor perturbation in the photophysical and $^1O_2$ generating properties of their excited states. At first sight the data for the complementary DEAEMA:HEMA polymers doped with TPPS (Table 3) appear to be similar to those of the TMPyP system. Under nitrogen-bubbled conditions the absorbance difference data for TPPS in DEAEMA:HEMA could be fitted to biexponential decays of approximately similar amplitude with lifetimes of ca. 400 and 1200 μs, the corresponding values for TMPyP were ca. 350 and 1300 μs. Similarly, the excited state lifetimes of TPPS in solution and pure HEMA (where no electrostatic binding is expected) both fell dramatically, as expected, when O_2 was bubbled through initially degassed samples, in both cases falling by ca. 300×, e.g., from 891 μs to 3 μs for HEMA.

Surprisingly, in view of the previous results, it was found that with 10−30% DEAEMA samples the excited state TPPS lifetimes were barely reduced at all on oxygen bubbling. For 10:90/DEAEMA:HEMA under standard loading conditions (5 immersions in 10 μg/mL of TPPS, absorbance at $\lambda_{max} \approx 2$) the two-component biexponential fit gave lifetime reductions of the two components of 1158 to 1100 μs and 497 to 303 μs and similar reductions were also observed for the 20:80 and 30:70 copolymer samples. Bearing in mind the ca. 300× lifetime reduction observed on O_2 bubbling of 0:100/DEAEMA:HEMA (i.e. pure HEMA) samples, it is remarkable that with addition of just 10% of the DEAEMA copolymer in 10:90/DEAEMA:HEMA the lifetime of the longer lived component was effectively unchanged on O_2 bubbling and the shorter component was reduced by a factor <2×. This suggests that in these

copolymers the sensitizer that gives rise to the longer component in degassed conditions is located in domains where O_2 quenching is effectively prevented either by slow diffusion or low solubility in those regions. The porphyrins which have a shorter lifetime under degassed conditions are obviously in a different chemical environment and this is also reflected in the small extent of quenching that is observed on O_2 bubbling. A difference in the O_2 quenching efficiency was also observed for the TMPyP system in that a nominally biexponential degassed sample gave a nominally biexponential quenched trace. However, for the TMPyP system O_2 bubbling led to very large lifetime changes, so that in 10:90/MAA:HEMA even the longer component in the quenched sample had a lifetime (38 μs) that was $>10\times$ shorter than the short-lived (488 μs) component of the degassed sample. Consistent with these observations, it was found that none of the TPPS/DEAEMA:HEMA systems gave detectable $^1O_2$ emission signals at 1270 nm, although the $\phi(^1O_2)$ for TPPS in solution is 0.67[17] and TPPS in 100% HEMA did give a weak signal, consistent with the significant lifetime reduction that is observed when it is O_2 bubbled. Of course it is possible that the first monolayer of TPPS in all these systems is open to oxygen quenching and here we have found evidence that even in the DEAEMA:HEMA systems the shorter lived component is quenched to a small extent on oxygen bubbling; however, our $^1O_2$ detection system was not sufficiently sensitive to detect the small quantities of $^1O_2$ that these processes might provide.

It is difficult to give a definitive reason for the remarkably low quenching efficiency of O_2 in the DEAEMA:HEMA systems but it is presumably associated with the very strong binding interactions between the TPPS and the host copolymer. The confocal fluorescence measurements (Figure 5) show that the TPPS initially binds at the exterior but, in contrast to the TMPyP system, further immersions in the doping solution do not result in the TPPS diffusing through the previously modified layer before ultimately binding deeper into the interior. Instead, even at very high doping levels the porphyrin remains confined within a thin surface layer. It is probable that it is strong cross-linking of the polymer chains by the polyanionic TPPS that reduces diffusion of TPPS through previously doped polymer regions. Similarly, such cross-linking may also be responsible for reducing the oxygen permeability of the doped TPPS films to such an extent that oxygen quenching is dramatically reduced. Previous studies on *meso*-sulfonatophenyl porphyrin covalently bonded to poly(vinyl alcohol) found that oxygen quenching, which was efficient in solution, was strongly reduced in dry porphyrin-modifed PVA films which have very low oxygen permeability.[12]

Release Kinetics. Since the intention is to use these materials in clinical applications it is important that the sensitizers remain bound to the polymer matrices for at least the length of time the antibacterial effect they exert is required, which is a few weeks at most for a material designed specifically to prevent postoperative infection. Release kinetics were recorded by immersing the porphyrin-doped polymer samples in buffer and withdrawing 1 mL aliquots at weekly intervals. Porphyrin concentration in the aliquots was then measured fluorimetrically ($\lambda_{ex} = 423$ nm, $\lambda_{em} = 685$ nm for the MAA:HEMA systems; $\lambda_{ex} = 413$ nm, $\lambda_{em} = 645$ nm for the DEAEMA:HEMA systems).

The data for the MAA:HEMA copolymers are summarized in Table 4, which gives values for the cumulative release. Consistent with the expectation that the TMPyP will be less strongly held in the 100% HEMA polymer than in the MAA-treated polymers, where electrostatic interaction is anticipated, the 0:100/MAA:HEMA polymer shows almost twice as much release as the 10−30% MAA copolymers. The release kinetics (not shown) are also different for the 100% HEMA and the 10−30% MAA polymers, in the former most of the 16% release is in the first week while in the latter systems there is a smaller initial release (ca. 4%) followed by a gradual increase to the final (10 week) value, which is still less than 10% of the bound porphyrin.

TABLE 4: Cumulative Release Data for TMPyP from MAA:HEMA Copolymers of Varying Composition[a]

MAA:HEMA composition	TMPyP released/%
30:70	9.2 ± 1.1
20:80	8.6 ± 0.5
10:90	7.4 ± 1.4
0:100	16.3 ± 1.9

[a] Data were measured fluorimetrically over a 10 week interval.

In contrast, negligible release was detected from the DEAEMA:HEMA copolymers suggesting that the porphyrin is very tightly bound within this polymer system, which is consistent with the confocal fluorescence measurements. Similarly, the large release from the 0:100/DEAEMA:HEMA copolymer ((23.9 ± 6.4)% over the 10 week period) is expected because no cationic groups were introduced to electrostatically bind the anionic porphyrin.

Conclusion

The general approach of preparing porphyrin-modified polymers by electrostatically binding charged porphyrins to copolymers which carry pendant groups of opposite charge has proved to be a remarkably straightforward and effective method of loading controlled amounts of material near the polymers' surfaces. Since hydrophilic, swellable polymers with a high water content were used it was expected that the photophysical properties of the bound porphyrins would be at least similar to those in simple aqueous solution. For TMPyP in MAA:HEMA copolymer matrices this was the case—the lifetimes of the excited triplet state porphyrins were slightly longer than the solution values and although biexponential (or pseudobiexponential) decay was observed this is not unusual for sensitizers bound in microheterogeneous hosts. Similarly, TMPyP in MAA:HEMA copolymers was strongly quenched by oxygen and the samples showed $^1O_2$ emission; this $^1O_2$ was generated at, or near, the surface (the porphyrin penetrated <200 μm) and its lifetime was similar to that in aqueous solution. In contrast, TPPS in DEAEMA:HEMA copolymers was unexpectedly resistant to oxygen quenching. In degassed samples the photophysical behavior was similar to that of TMPyP but introduction of oxygen had only a very small effect on the triplet lifetimes and no $^1O_2$ emission could be detected. This unusual behavior appears to be associated with the strong binding of the porphyrin to the host which, although it does not perturb the photophysical properties of the porphyrin per se, does restrict access by oxygen. This effect means that the TPPS/DEAEMA:HEMA system is a less promising candidate as a postoperative antibacterial treatment for intraocular lenses than TMPyP in MAA:HEMA in which the porphyrin is localized at the surface but retains its ability to generate $^1O_2$ on photoexcitation.

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(16) Footnote: The shorter triplet lifetime in N_2-bubbled solution compared to the equivalent value in polymer may be due to residual O_2 that can effectively quench solution phase porphyrin but is less effective in the polymer—an effect that can also account for the differences in lifetime in air-saturated samples which were 2 ms in solution and 500 ms in polymer. The short solution-phase lifetime is not due to triplet−triplet annihilation since reduction in the photolysis pulse energy (1 to 0.25 mJ) did not change the measured lifetime.

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