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Bacterial adhesion to phosphorylcholine-based polymers with varying cationic charge and the effect of heparin pre-adsorption

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The steady increase in the use of medical implants and the associated rise of medical device infections has fuelled the need for the production of biomaterials with improved biocompatibility. 2-(methacryloyloxyethyl phosphorylcholine) (MPC) based coatings have been used to improve the biocompatibility of a number of different medical devices. Recent studies have investigated the use of a phosphorylcholine modified with cationic charge to encourage specific bio-interaction. Until now the affect of cationic charge incorporation in MPC copolymers on bacterial adhesion has not been investigated. This study attempts to address this by investigating the affect of charge on four different strains of bacteria commonly associated with medical device infections. In addition, the affect of pre-incubating these MPC-copolymers in heparin is also evaluated as this has previously been shown to improve biocompatibility and reduce bacterial adhesion. Bacterial adhesion was assessed by ATP bioluminescence and Scanning Electron Microscopy (SEM). Results suggest that bacterial adhesion generally increased with increasing cationic charge. When samples were however, pre-incubated with heparin a significant reduction in bacterial adhesion to the MPC-based samples was observed. The heparin remained bound and effective at reducing bacterial adhesion to the cationic MPC-based samples even after three weeks incubation in PBS. To conclude, the MPC-based cationic polymer coatings complexed with heparin may provide a promising solution to reduce medical device related infections.

Introduction

Bacterial infection is still a major problem associated with medical devices and is often the cause of device failure [1]. Following the implantation of a device there is a high risk of infection as damaged tissues, caused during surgery and an up-regulated inflammatory response, provide a portal of entry for potential pathogens. The propensity of a device to support bacterial colonisation subsequently affects the longevity of the implant.

Bacterial adhesion is an essential step in the pathogenesis of infection. For a micro-organism to colonise a biomaterial it must first physically approach and make contact with it. Generally, two types of interactions govern bacterial attachment to a surface. The factors involved in the initial adhesion of bacteria to a substrate can be explained in terms of non-specific interactions (electrostatic forces, hydrogen bonds and Van der Waals forces) and hydrophobic interactions. Van der Waals forces which are usually attractive, come in to play at a separation distance (between bacteria and surface) of >50 nm and hold the bacteria relatively weakly to the surface. According to Derjaguin, Verwey, Landau and Overbeek (DVLO) theory [2, 3] this is the secondary energy minimum, where a larger separation distance means the bacterium is still easily removed by shear forces [4]. At a separation distance of about 10–20 nm, the bacterial cell, although weakly held, is kept away from the substrate surface by electrostatic repulsion forces. Electrostatic interactions can be attractive if the surfaces are oppositely charged. Bacteria however, are usually negatively charged due to cell constituents, which contain phosphate, carboxyl and acidic groups. Potential attachment surfaces also tend to be negative due to conditioning by organic materials in the surrounding environment or its chemistry. As such, electrostatic interactions are usually repulsive between bacteria and a substrate surface. At shorter distances, the repellent electrostatic forces increase due to an overlap of the electron clouds of both bacteria and surface. This (electron cloud) is affected by the ionic strength

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of the surrounding medium; as ionic strength increase, the repulsive nature of the electrostatic force decreases. Most natural environments have high ionic strength due to the presence of electrolytes and as such, tend to counteract these repulsive forces. At <2 nm, water adsorbed to bacteria or substrate surfaces can act as a barrier to bacterial attachment. The exclusion of water to enable attachment is not kinetically favourable, hydrophobic interactions however, if present (usually within 2 nm of the surface) can help exclude water through non-polar regions on both surfaces. Once a separation of <1 nm is reached, other adhesion forces such as ionic bridging, hydrogen bonding and ligand-receptor interactions occur. According to the DVLO theory, the primary minimum energy is reached at this stage and the forces are strong enough to enable specific, short-range interactions. At this stage specific bacterial adhesins like fimbrae mediate adhesion [5]. Once attached to the surface bacteria often produce an extra cellular polysaccharide substance (EPS), this results in the formation of a biofilm. The EPS reinforces the binding, making it difficult to remove the bacteria, whilst providing nutrient and protection from phagocytosis, antibodies and antibiotics [1, 6, 7]. In such a system the bacteria can multiply and establish an infection that can not be controlled by the host.

Much research has been carried out over the years to reduce infection of medical devices, with one of the major emphases being on the creation of materials which inhibit initial bacterial adhesion and colonisation. One method used to improve a material's biocompatibility is the use of polymer coatings. Phosphorylcholine (PC) Technology^{TM} is a family of polymers that have been developed to mimic one of the phospholipid headgroups found in the biological membrane [8]. The seminal studies demonstrating the biocompatibility of phosphorylcholine and the synthesis of phosphorylcholine containing polymers was initially carried out by Chapman's group in the UK and Nakabayashi's group in Japan in the 1980's [9, 10]. Many studies have since been carried out on MPC-coated biomaterials proving its biocompatibility: MPC-based copolymers have been shown to reduce platelet adhesion and activation on material surfaces [11]. This is probably due to a reduction in protein adhesion, especially those involved in the coagulation cascade such as fibrinogen and factor XII [12, 13]. Lloyd *et al*. [14, 15] also showed that MPC-based materials significantly reduced bacterial adhesion, human macrophage and granulocyte adhesion, mouse fibroblast and rabbit lens cell adhesion.

Recent studies have suggested that MPC-based coatings may be modified to combine the biocompatible properties of MPC with more bio-interactive properties. The incorporation of charge on a material's surface has previously been shown to increase protein adsorption [16], alter protein conformation [17] and increase cell adhesion [18, 19]. Cationically modified MPC copolymers containing choline methacrylate (CMA) in amounts varying from 0 to 30% have been synthesised and fully characterised, the details of which may be found in Lewis *et al*. (2004) [20]. Rose *et al*. (2004) [21] and Palmer *et al.* [22] showed that protein adsorption and cell adhesion on these samples could be controlled by varying the cationic charge in these MPC-based coatings. As the effect on bacterial adhesion to these materials has not been assessed this study investigates the affect of varying cationic charge on adhesion of a number of bacteria commonly associated with medical device infections.

In addition, heparin, which is known for its antithrombogenicity and its strong negative charge that repels cellular organisms, was complexed with the coatings and its ability to reduce bacterial adhesion was assessed. Previous studies have demonstrated that heparin reduces bacterial adhesion; Rugieri *et al.* [23], showed a 90% reduction in bacterial adhesion on urinary catheter surfaces coated with heparin; Tenke *et al*. (2004) [24] showed that heparin-coated ureteral stents protect against encrustation and biofilm formation for 6–12 months, both *in vitro* and *in vivo*. The bacterium *Escherichia coli* was chosen for these additional studies as it is commonly associated with urological device infection; *Escherichia coli* accounts for nearly 13% of all catheter-associated urinary tract infections (CAUTIs) [25].

Methodology

Preparation of MPC coated samples

MPC-based polymers with varying amounts of cationic charge were prepared and supplied by Biocompatibles UK Ltd, full synthesis and characterisation of these polymers are described in Lewis *et al*. [20]. Poly (ethyleneterephthalate) (PET) strips were dip coated in 10mg/ml solutions (in ethanol) of each of the MPC-based polymers. The coated strips were cured at 70 °C for 72 h and then gamma irradiated to ensure cross linking of the polymer to the PET substrate. Prior to bacterial adhesion assessment, 1 cm^2 samples of the coated PET were cut and sterilised under UV for 2 h.

Bacterial strains

Staphylococcus epidermidis 901B is a clinical isolate obtained from infected dialysis fluid obtained from a CAPD (Continuous Ambulatory Peritoneal Dialysis) patient and donated by the renal unit, City Hospital Nottingham. *Pseudomonas aeruginosa* 10548, *Staphylococcus aureus* 10788 and *Escherichia coli* 8196 are commercially available organisms obtained from the National Collection of Type Cultures (NCTC). For the duration of this study, cultures of the above organisms were maintained on Tryptone Soya Agar (TSA) plates stored at 4 °C.

ATP assay

The bioluminescent ATP assay has been used by a number of different groups to assess bacterial adhesion of materials [26, 27]. The ATP assay is an indirect method of measuring bacterial numbers, quantifying the number of cells by extracting their ATP. All cells are dependent on ATP to stay alive sand the intracellular level of

ATP is precisely regulated, on cell death ATP is very rapidly lost and levels decline [28]. This technique is based on the quantitative measurement of light produced as a result of an enzyme (luciferase) catalysed reaction.

$$\mathrm{ATP} + \mathrm{Luciferin} + \mathrm{O_2} \overset{\mathrm{Luciferase}}{\Longrightarrow} \mathrm{oxyluciferin} + \mathrm{AMP} + \mathrm{PPi} + \mathrm{CO_2} + \mathrm{Light}$$

When other components of the reaction remain constant, the intensity of light emitted is proportional to the amount of ATP taking part in the reaction. By use of a standard curve based on known concentrations of bacteria the actual number of cells can therefore be calculated. This method is especially useful in microbiology for counting bacteria which are adherent to surfaces.

A single colony of bacteria from an agar plate was used to inoculate 100 mL of typtone soya broth (TSB) and incubated overnight in a shaking incubator set at 37 °C. After incubation the culture was centrifuged, washed and finally re-suspended in PBS. The bacterial suspension was diluted to approximately 10^8 cells/mL. 1 mL of the diluted bacterial suspension was added to each sample in 24 well plates. The samples were then incubated at 37 °C with gentle agitation for 3 h. After incubation each sample was removed from the 24 well plates and washed in sterile PBS before placing in a clean 24 well plate containing 300 μL of bactolyse (Cambrex, UK) to lyse the cells. The samples were then incubated for 15 minutes at room 20 °C with gentle agitation. In addition, dilutions of the washed bacterial overnight cultures were prepared, centrifuged, resuspended in bactolyse and incubated as above, to provide a standard curve for each bacterial type. After incubation, 100 μL of each bactolysed solutions were pipetted (in duplicate) into a white 96 well plate. Plates were then assessed in the Athos Lucy 1 luminometer (Anthos Labtech Instruments, UK) to determine the luminescence of each sample solution following addition of ATP monitoring regent. The number of cells (cells/cm^2) adhered to each sample were calculated from the standard curve obtained from the readings of the diluted bacterial culture and from viable count plates prepared from dilutions of the washed overnight cultures.

SEM

Samples were incubated with bacteria as above and then rinsed three times in PBS before fixing in 2.5% glutaraldehyde. After fixing, the samples were dehydrated in a series of ethanol solutions increasing in concentrations; 30, 50, 70, 90 and 100% for 15 minutes each concentration. Samples were then sputter coated with palladium prior to imaging under SEM.

Heparin pre-adsorption study

Samples were pre-incubated in 40 U/mL of heparin for 1 hour at 37 °C prior to assessment of bacterial adhesion. Following heparin incubation samples were rinsed three times in sterile PBS. For the long term study, the samples were then incubated in PBS at 37 °C for three weeks (changing the PBS twice a week) prior to bacterial incubation. Samples were then incubated with *Escherichia coli* and bacterial adhesion was assessed using the ATP assay and SEM as discussed above. Samples that had not been pre-incubated in heparin and/or incubated in PBS were used as controls.

Results

Adhesion of *Pseudomonas aeruginosa* 10548 to cationic MPC polymer coatings.

Fig. 1(a) suggests that the adhesion of *Pseudomonas aeruginosa* 10548 was affected by cationic charge when the amount of choline in the MPC-based coatings was at or above 20%. An increase in bacterial adhesion was observed on the MPC 30% samples compared to the MPC coating with less than 20% charge and the uncoated samples (PET). When the amount of choline (charge) was less than 20% in the MPC-based coatings no difference was observed in the number of adherent bacteria. No difference was observed either, between these MPC-based coated samples and the uncoated PET. These observations are supported qualitatively by SEM shown in Fig. 1(b). Fig. 1(b) does suggest however, that *Pseudomonas aeruginosa* clumped together on the uncoated PET whereas on the MPC coated samples the bacteria adhered as separate cells.

Adhesion of *Staphylococcus epidermidis* (901B) to cationic MPC coated samples

Fig. 2(a) suggests that no decrease in bacterial adhesion was observed on the MPC 0% samples compared to the uncoated PET. The presence of a cationic charge in the MPC-based coating appeared to affect the adhesion of *Staphylococcus epidermidis* 901 when choline was present at concentrations as low as 10% (MPC10%);No difference in bacterial adhesion was observed between MPC 0% and MPC 5%, however when the choline content was 10% or greater in the MPC samples an increase in bacterial adhesion was observed compared to MPC 0 and 5%. When choline concentration increased above 10% a general increase in bacterial adhesion was observed. These observations are supported qualitatively by SEM in Fig. 2(b). In addition, the SEM images suggest that on the uncoated PET and the MPC 30% samples, *Staphylococcus epidermidis* started to aggregate together rather than adhering as separate cells.

Adhesion of *Staphylococcus aureus* (10788) to cationic MPC coated samples

Fig. 3(a) suggest an increase in adhesion of *Staphylococcus aureus* as the choline content in the MPC -based coatings increased to 15% and thereafter decreased. These observations are supported qualitatively by SEM in Fig. 3(b). The data obtained showed a high level of variability on some of the samples, this high variability may be due to the tendency of *Staphylococcus aureus* to aggregate together. The ATP assay suggests that no reduction in adhesion was observed on MPC 0% compared to the uncoated PET samples, however the SEM

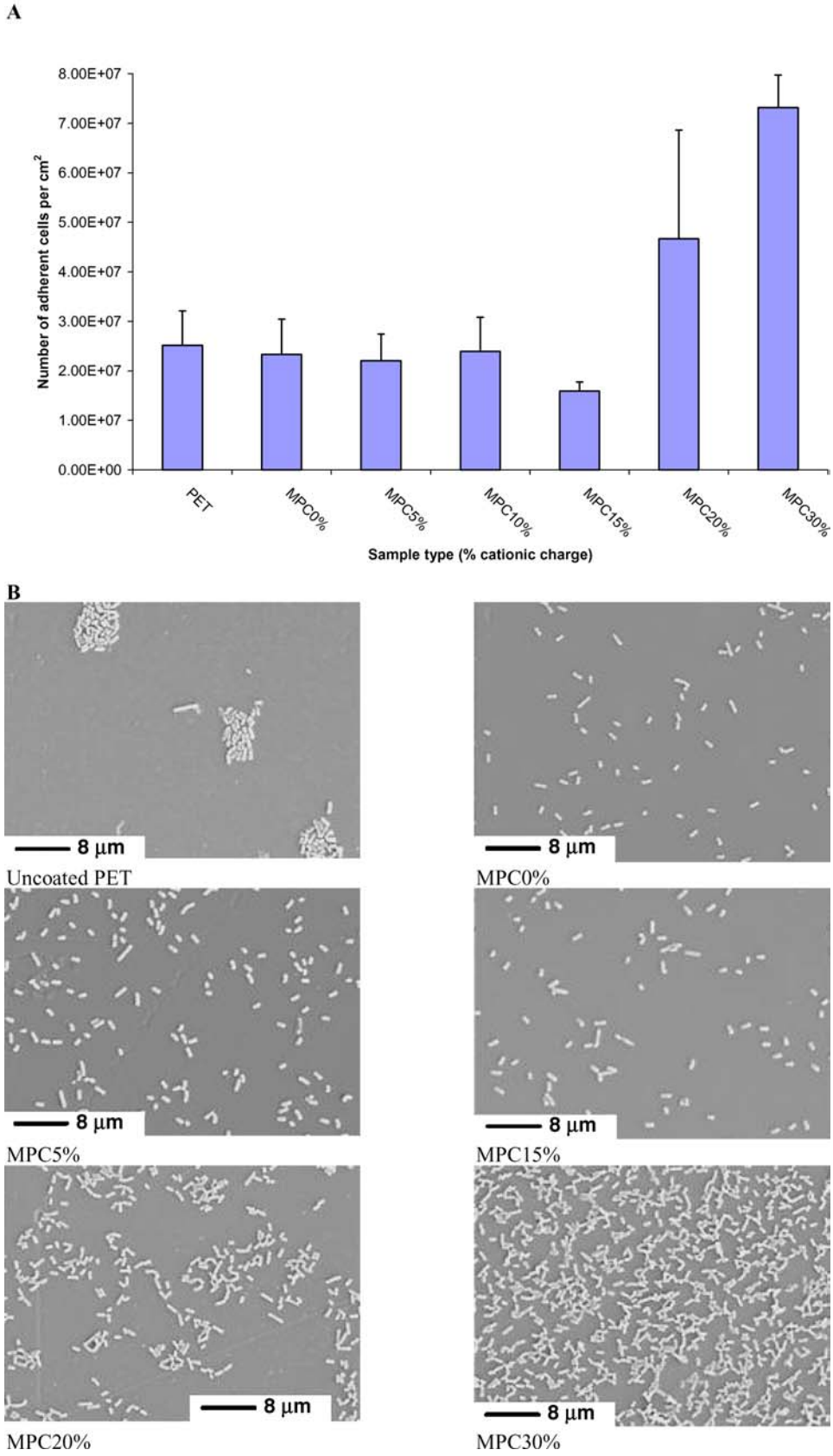

Figure 1 *Pseudomonas aeruginosa* 10548 adhesion to uncoated PET and MPC coated PET samples of varying cationic charge. MPC cationic samples were incubated in 8×10^7 cells/mL for 3 h at 37°C. (a) Graph showing mean numbers of adherent cells ± SD ($n = 3$) assessed using the ATP assay. (b) Representative photographs of adherent *Pseudomonas aeruginosa* 10548 cells on MPC cationic samples incubated with 8×10^7 cell/mL 3 h.

images suggest a decrease. The SEM images also suggest that *Staphylococcus aureus* started to clump together when the choline content in the MPC was at or above 20%.

Adhesion of *Escherichia coli* (8196) to cationic MPC coated samples

In a similar manner to that observed for *Staphylococcus epidermidis*, Fig. 4(a) shows that the adhesion

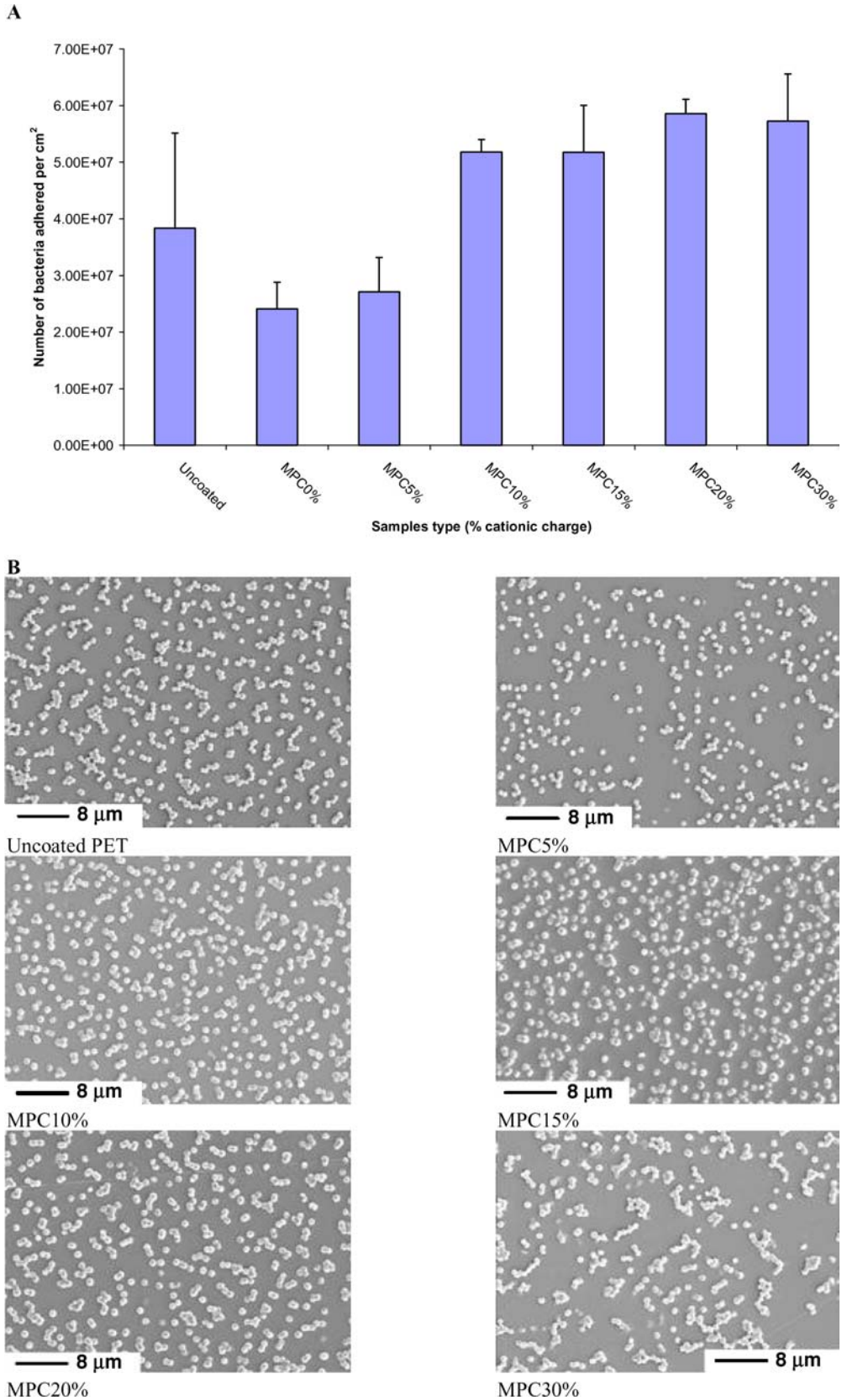

Figure 2 Staphylococcus epidermidis 901 B adhesion to uncoated PET and MPC coated PET samples of varying cationic charge. MPC cationic samples were incubated in 1.46×10^8cells/mL for 3 h at 37 °C. (a) Graph showing mean numbers of adherent cells ± SD ($n = 3$) assessed using the ATP assay. (b) Representative photographs of adherent *Staphylococcus epidermidis* 901 B cells on MPC cationic samples incubated with 1.46×10^8 cells/mL for 3 h.

of *Escherichia coli* is affected by cationic charge when the amount of choline is at or above 10% in the MPC -based coatings. No difference in bacterial adhesion was observed between MPC 0% and MPC 5%, however when the choline content was 10% or greater an increase in bacterial adhesion compared to MPC 0 and 5% was observed. A general increase in bacterial adhesion was observed as choline concentration increased above

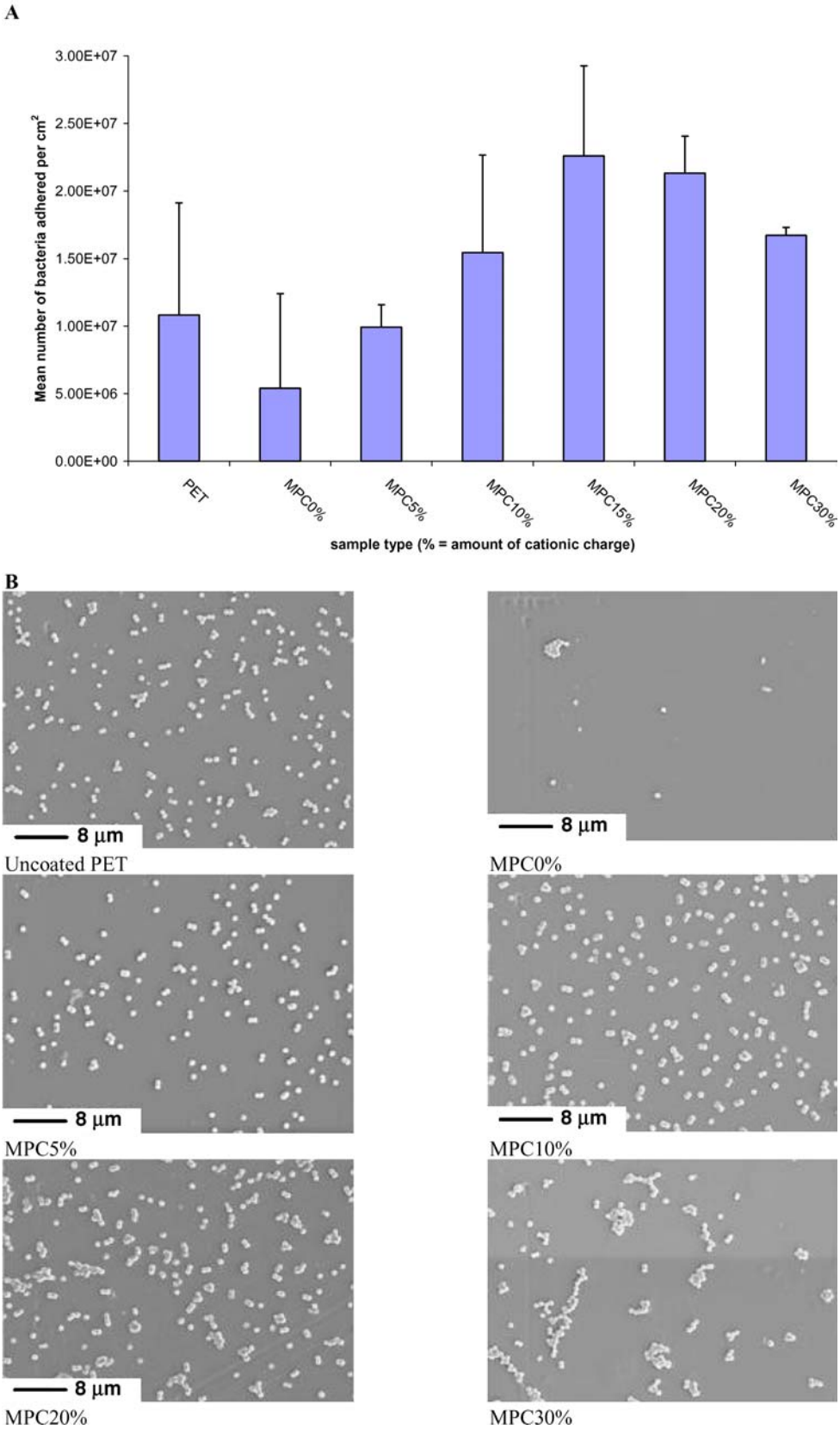

Figure 3 Staphylococcus aureus 10788 adhesion to MPC cationic samples. MPC cationic samples were incubated in 8.8×10^7cells/mL for 3 h at 37 °C. (a) Graph showing mean numbers of adherent cells ± SD ($n = 3$) assessed using the ATP assay. (b) Representative photographs of adherent *Staphylococcus aureus* 10788 cells on MPC cationic samples incubated with 8.8×10^7 cells/mL for 3 h.

10%. A decrease in adhesion as observed on MPC 0% compared to the uncoated PET samples, suggests that MPC with no charge reduces bacterial adhesion. These observations are supported qualitatively by SEM (Fig. 4(b)).

Differences in adhesion to the MPC cationic samples between bacterial strains

Fig. 5 suggests that the degree of bacterial adhesion is dependent upon the bacterial type since they do not all show the same trends. *Pseudomonas aeruginosa*

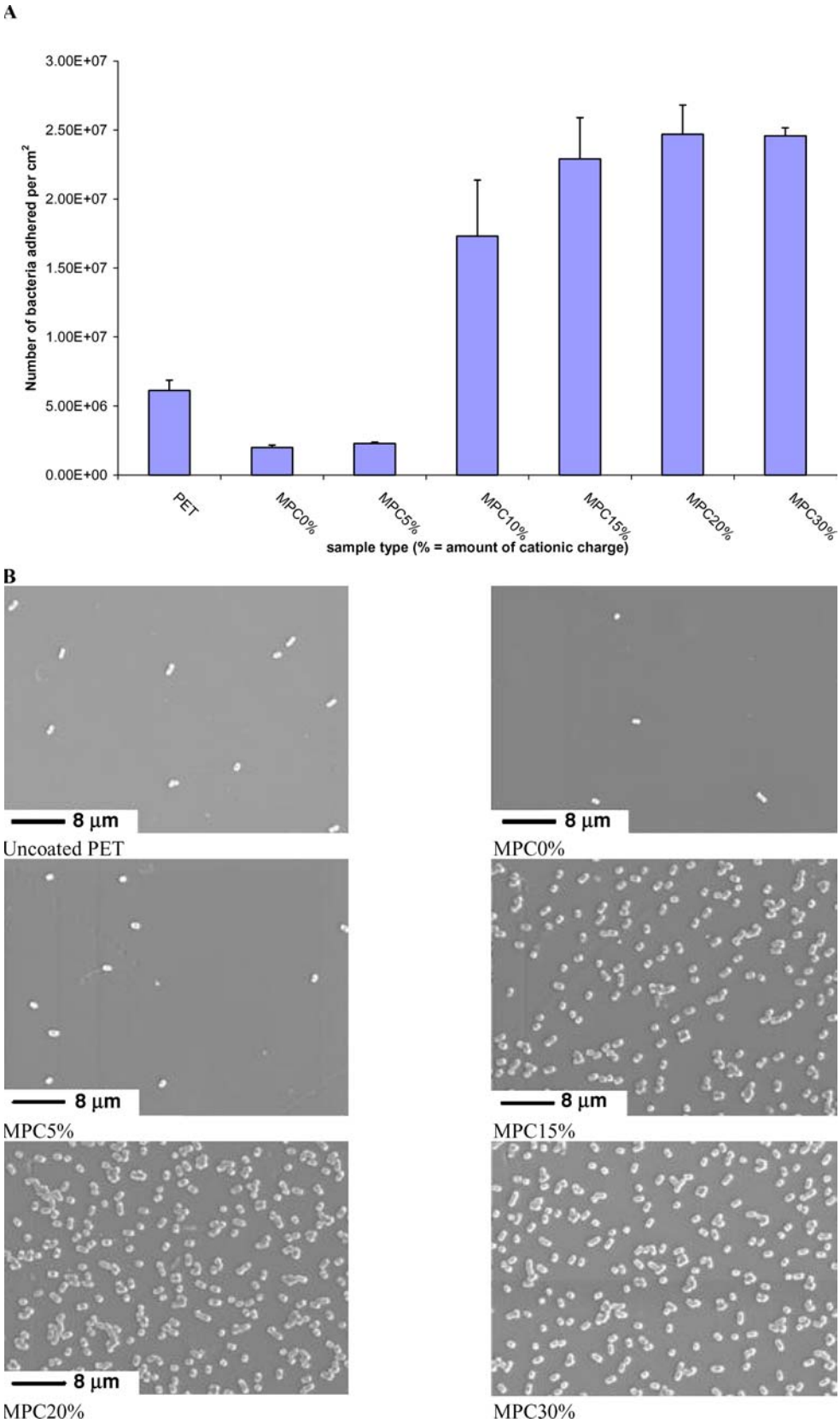

Figure 4 Escherichia coli 8196 adhesion to uncoated PET and MPC coated PET samples of varying cationic charge. MPC cationic samples were incubated in cells/ml 1.8×10^8cells/mL for 3 h at 37 °C. (a) Graph showing mean numbers of adherent cells ± SD ($n = 3$) assessed using the ATP assay.(b) Representative photographs of adherent *Escherichia coli* 8196 cells on MPC cationic samples incubated in a bacterial suspension of 1.8×10^8 cells/ml for 3 h.

generally adhered to the samples at a higher percentage of inoculum than the other bacterial strains; this was followed by *Staphylococcus epidermidis*, *Staphylococcus aureus*, with *Escherichia coli* adhering the lowest percentage of inoculum. *Staphylococcus epidermidis* and *Escherichia coli* show a similar pattern of adhesion as cationic charge increased; an increase in adhesion was observed when the MPC copolymer contained

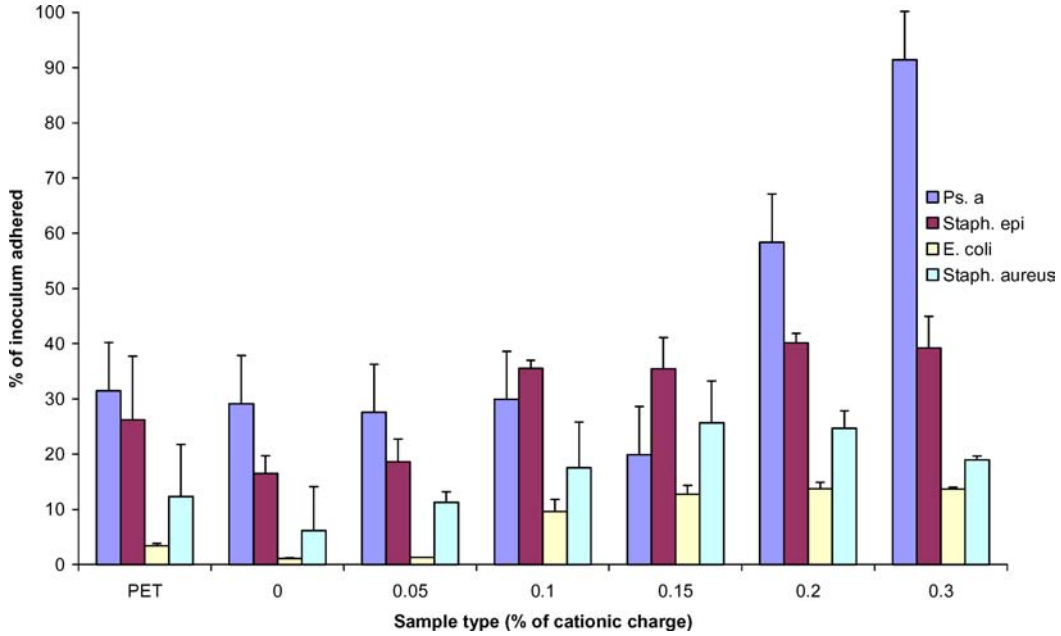

Figure 5 Percentage of bacterial inoculum adherent to MPC cationic samples. The MPC cationic samples were incubated with four different bacteria types at an approximate concentration of 1×10^8cells/mL for three h at 37 °C. Mean ± SD ($n = 3$).

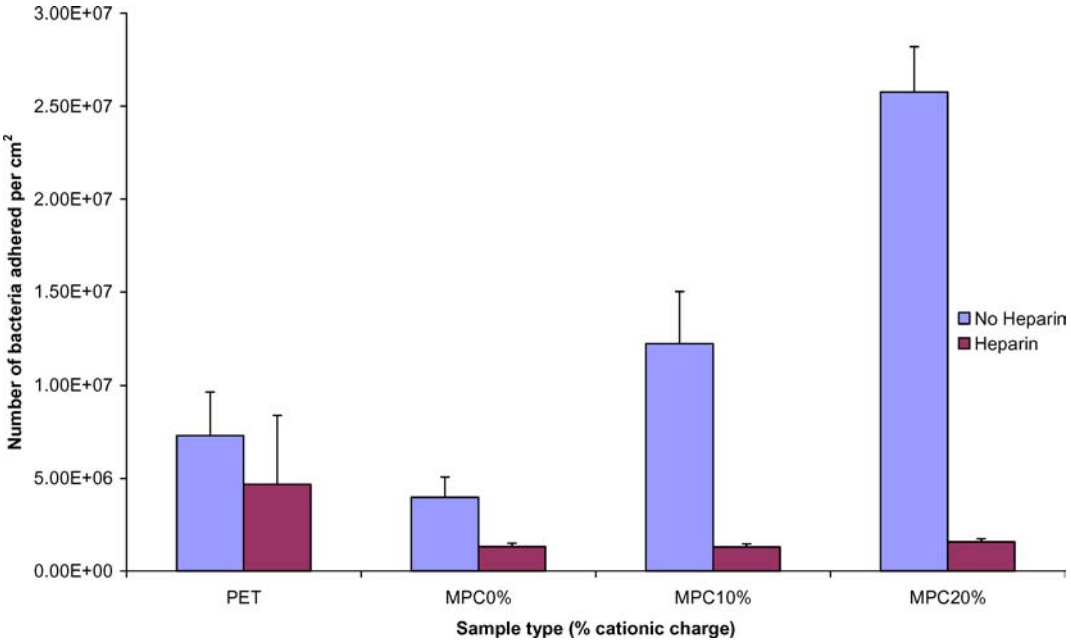

Figure 6 The effect of heparin pre-adsorption on *Escherichia coli* 8196 adhesion to uncoated PET and MPC coated PET samples of varying cationic charge. Half of the samples were pre-incubated with heparin for 1 hour at 37 °C prior to incubation with bacteria. Samples were incubated with 2.15 $\times 10^8$cells/mL for 3 h at 37 °C. The numbers of adherent cells were assessed using the ATP assay. Mean ± SD ($n = 3$).

a minimum of 10% choline, above 10% no further increase in adhesion was observed. *Pseudomonas aeruginosa* only showed an increase in adhesion when the choline content in the cationic MPC copolymer samples was above 20%. *Staphylococcus aureus* on the other hand, generally increased in adhesion as choline content increased up to 15% and then generally decreased as choline content increased above that.

The effect of heparin pre-adsorption on adhesion of *Escherichia coli*

Figs. 6 and 7 shows that pre-incubation of samples in heparin reduced the adhesion of *Escherichia coli* to all of the MPC copolymer coated samples. As observed previously, bacterial adherence increased as cationic charge increased on those samples that had not been pre-incubated in heparin. No difference in bacterial adhesion was observed between the MPC copolymers that had been pre-incubated with heparin as cationic charge increased.

The long term affects of heparin on adhesion of *Escherichia coli*

Figs. 8 and 9 suggests that heparin is still effective at reducing *Escherichia coli* adhesion to cationic MPC coated samples even after three weeks incubation in

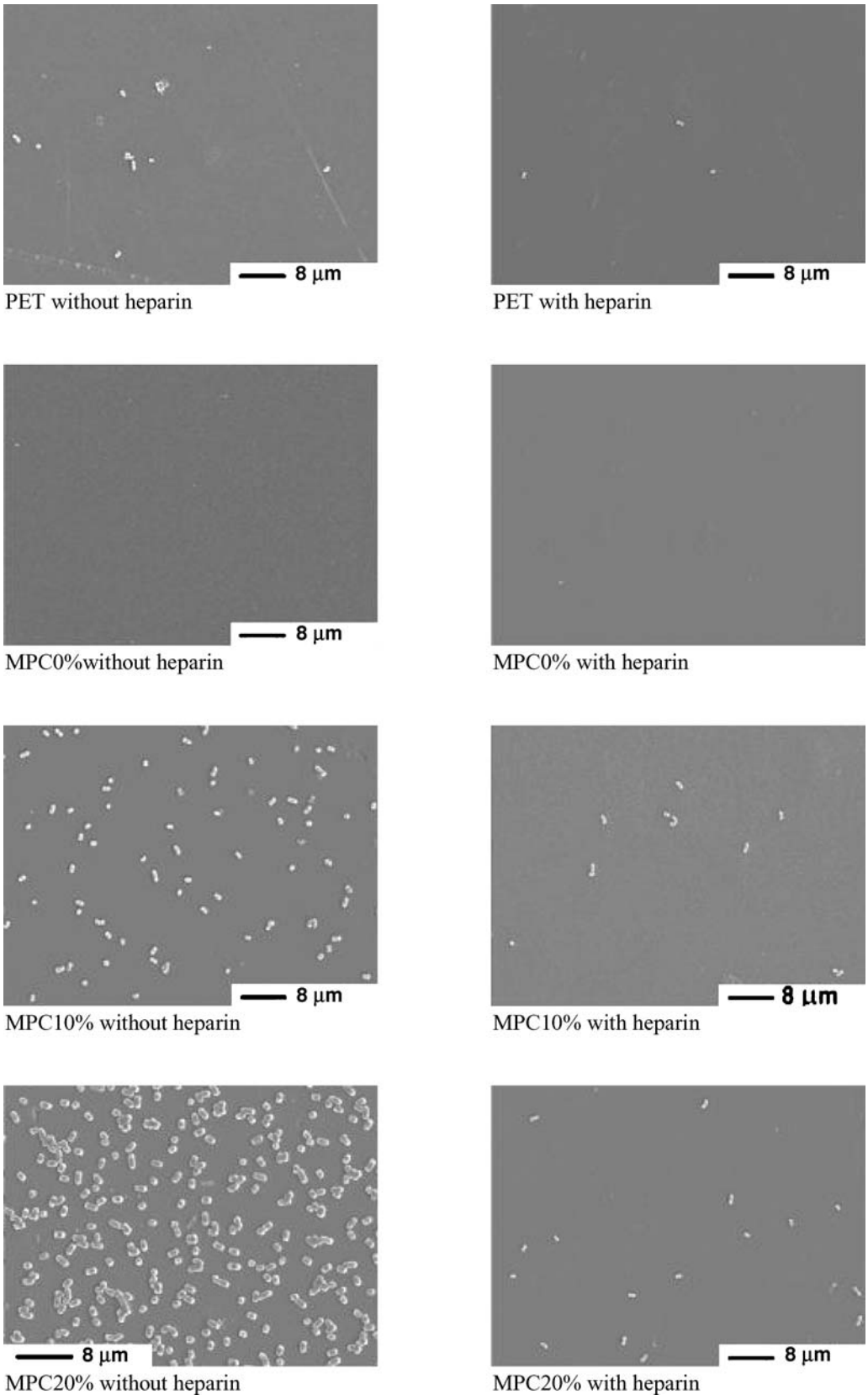

Figure 7 Representative photographs comparing *Escherichia coli* 8196 adhered to samples pre-incubated and not pre-incubated in heparin prior to incubating in bacteria for 3.

PBS; MPC 10 and 20% coated samples pre-incubated in heparin reduced bacterial adhesion after 3 weeks in PBS. No difference in bacterial adhesion was observed however, on the MPC copolymers with no charge (MPC 0%) pre-incubated in heparin compared to the samples not pre-incubated in heparin.

The MPC copolymer coatings that were not pre-incubated in heparin but incubated in PBS for three weeks adhered more bacteria than the no heparin/no PBS MPC copolymer coating. No difference in bacterial adhesion was observed on the uncoated PET samples (without heparin) that had been incubated in PBS

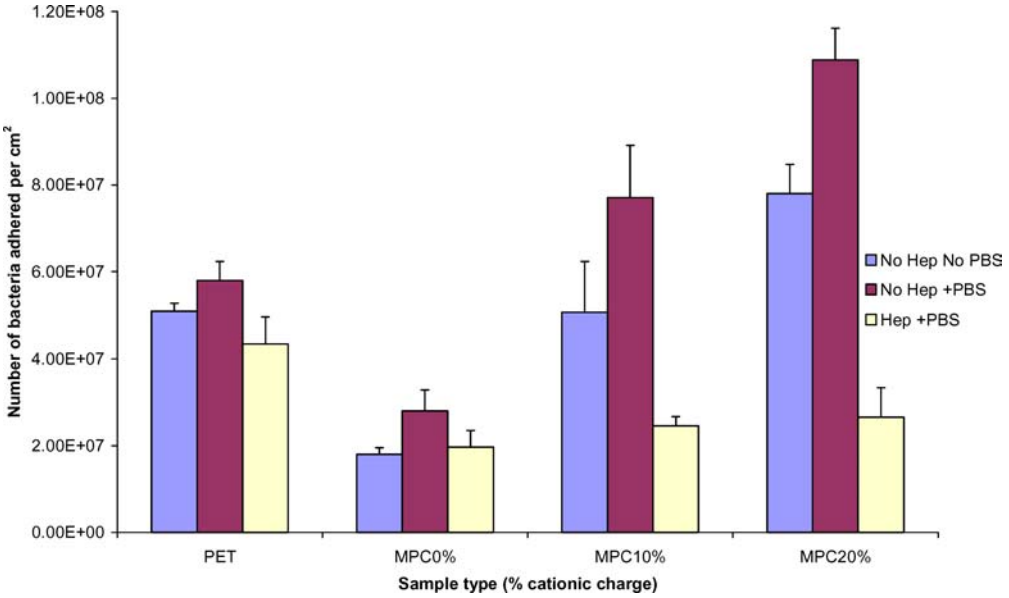

Figure 8 The effect of heparin on adhesion of *Escherichia coli* 8196 to uncoated PET and MPC coated PET samples of varying cationic charge after three weeks. A third of the samples were not pre-incubated in heparin or incubated with PBS (No Hep No PBS), a third of the samples were incubated in PBS for three weeks at 37 °C (No Hep +PBS) and a third of the sample were pre-incubated in heparin for 1 hour at 37 °C and then incubated in PBS for three weeks. Samples were incubated with 2.42×10^8cells/mL for 3 h at 37 °C. The numbers of adherent cells were assessed using the ATP assay. Mean ± SD ($n = 3$).

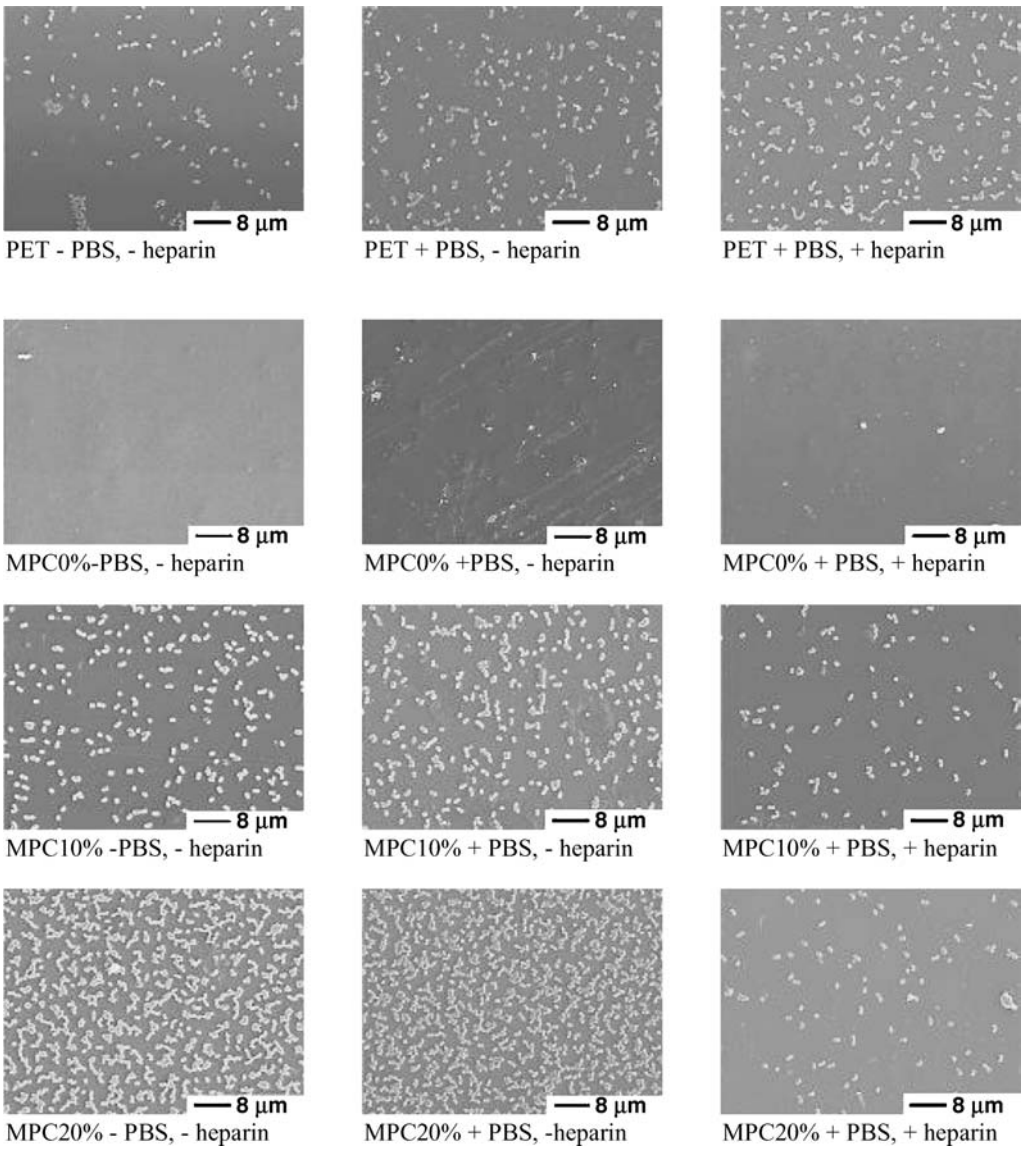

Figure 9 Representative photographs comparing *Escherichia coli* 8196 adhered to samples pre-incubated and not pre-incubated in heparin and incubated and not incubated in PBS for three weeks prior to incubating in bacteria for 3.

compared to those that had not been, suggesting that PBS has no effect on bacterial adhesion to PET.

Discussion

Bacterial adhesion

Since MPC-based materials have been reported to confer reduced bacterial adhesion [13], a reduction in bacterial adhesion was expected between the uncoated PET sample and MPC 0%. However, this was not the case for all four bacterial strains in this study. A significant decrease in adhesion was observed in the ATP assay results for *Escherichia coli* which were confirmed in the SEM images. No significant decrease in adhesion was observed for the other bacterial strains in the ATP assay however; the SEM images for *Staphylococcus aureus* suggest that MPC 0% reduced bacterial adhesion compared to the uncoated PET. No Significant difference in adhesion was observed between the uncoated PET and MPC 0% samples for *Staphylococcus epidermidis*; this is probably due to the high variation observed between replicate uncoated PET samples. The uneven adhesion due to *Pseudomonas aeruginosa* clumping on the uncoated PET samples (as observed by SEM) could explain the unexpected lack of reduction on MPC 0% observed with this bacterial strain.

The results obtained from the bacterial adhesion assay without pre-incubation in heparin showed an increase in bacterial adhesion when a cationic charge (choline) was present in the MPC-based coating. The percentage of choline required to significantly increase bacterial adhesion however, was specific to the bacterial strain. This increase in bacterial adhesion on the cationically charged surfaces is most likely a result of electrostatic attraction between the negatively charged surface of the bacteria and the cationically modified material. The DVLO theory [2, 3] explains this long range interaction between bacteria and substrate surfaces in terms of attractive and repulsive forces. In the past, other groups [29] have shown that initial bacterial adhesion to positive surfaces is higher compared with negative ones and as such, support our findings.

The results obtained in this study were similar to that observed by Rose *et al*. [21] for mammalian cells; an increase in adhesion was observed with the addition of charge in the MPC-based coatings, although the response was non-linear as charge increased. In this study the results clearly show that bacterial adhesion is affected by charge, however, a linear relationship was not observed for all four bacteria. These results would therefore suggest that factors other than material surface charge effects bacterial adhesion. Such factors could include bacteria and materials physico-chemical surface properties; Bruinsma *et al*. [30] demonstrated that hydrophilicites varied between bacterial strains, with the more hydrophobic strains adhering more extensively to contact lenses than the hydrophilic bacteria. Satou *et al*. [31] found that bacterial adhesion to glass was related to the physico-chemical surfaces properties of individual strains; hydrophobic bonding was more important to some bacterial strains whereas ionic interactions made the highest contribution to adhesion for other bacteria. The materials surface hydrophilicity as well as surface charge is thought to influence bacterial adhesion; Garcia-Saenz *et al*. [32] found that the more hydrophilic the intraocular lens (IOL), the less bacteria adhered. Triandafillu *et al*. [33] showed that hydrophilisation of poly(vinyl chloride) (PVC) using oxygen-plasma treatment significantly decreased initial adhesion of bacteria. Lewis *et al*. [20] found that the hydrophilicity of the MPC copolymer coating increase as choline content (charge) increased; this may help to explain the non-linear bacterial adhesion response to increasing cationic charge. It is also possible that the different strains of bacteria have varying hydrophilic preferences for adhesion, which could contribute to the differing pattern of adhesion between bacterial strains observed here; Triandafillu *et al*. [33] found that adhesion to PVC varied widely even between different strains of the same species.

A previous study carried out by Kishda *et al*. [18], although looking at mammalian cells, has suggested that increased electrostatic interactions with increasing charge can be strong enough to cause cells to detach. This is a possible explanation for the reduction in adhesion at the highest cationic charge as observed for *Staphylococcus aureus*. This theory may also explain the reduced rate of increase in adhesion for *Escherichia coli* and *Staphylococcus epidermidis* at the higher cationic charges. Although this study was limited to the initial adhesion of bacteria to the surface of the samples, it is possible that cationic charge might also have an effect on bacterial growth; previous studies have suggested that a high positive surface charge might exert a strong adhesive force on negatively charged bacteria, which has been proposed to physically inhibit surface growth of rod-shaped bacteria [34], further studies would be required to assess this hypothesis on these samples. It is also possible that these strong electrostatic interactions have resulted in aggregation of *Staphylococcus epidermidis* and *Staphylococcus aureus* at the higher cationic charges as observed by SEM.

Comparisons in adhesion between bacterial types revealed that *Pseudomonas aeruginosa* generally showed the highest percentage of inoculum adhered. Andrews *et al*. [26] showed similar findings when investigating the adhesion of a number of bacteria genera to contact lenses; they found that *Pseudomonas aeruginosa* showed greatest adhesion compared to *Staphylococcus epidermidis* and *Serratia marcescens*. The production of large amounts of the exopolysaccharide (EPS), alginate, by *Pseudomonas aeruginosa* [35] might contribute to this increase in adhesion. These observed differences in adhesion between bacterial types could be due to differences in bacterial surface properties; it is possible that the relatively highly electronegative lipopolysaccharide membrane of *Pseudomonas aeruginosa* [36] might enable a higher degree of electrostatic interaction. A previous study by Gottenbos *et al*. [29] reported that gram-positive strains have less extensive contact with positively charged surfaces when compared to gram-negative strains even under conditions of electrostatic attraction. This is thought to be a result of

the relatively thicker peptidoglycan layer of gram positive cells. The results obtained here do not however, support this suggestion as *Staphylococcus epidermidis* and *Staphylococcus aureus* both had a higher percentage of inoculum adhered to the MPC cationic samples than the gram negative bacterium, *Escherichia coli*.

The affect of heparin pre-incubation

Several studies have previously demonstrated that heparinisation of blood contacting devices reduces the risk of thrombus and infection [24, 32, 37–39]. Results from this study confirm the ability of heparin to reduce bacterial adhesion, thus suggesting that heparinisation of cationic MPC copolymers could potentially reduce the risk of infection if used to coat medical devices. These results suggest that this inhibitory effect is stable as bacterial adhesion was reduced on MPC cationic samples even after being incubation in PBS for three weeks following heparin adsorption. It is suggested that the heparin, being strongly electronegative [24], binds to the positive charge on the MPC coating. It is thought that a like for like repulsion force between the bacterial cells, which are generally negatively charged, and the heparin inhibits bacterial adhesion. Additionally, heparin-modified surfaces are thought to hydrate the surface which in turn is thought to modify some structural fatty acids on the bacteria surface and thus reduce bacterial adhesion [32, 40–42].

Comparisons between the samples that were and those that were not incubated in PBS without heparin, suggest that PBS increased bacterial adhesion to the MPC-based polymer coated samples. It is possible that PBS changes the swelling (water content) of the MPC-based coating. The ionic interactions between the charged polymer bound groups and the ionic media (PBS) may reduce the potential of the coating to swell and thus reduce the water content. Durmaz and Oakey [43] showed that the degree of swelling was stabilised within charged polymers that were exposed to ionic media. Previous studies have demonstrated that bacterial adhesion decreases with increase in material surface hydrophilicity [32, 33]. The presence of heparin in combination with PBS however, appears to counteract the effect that PBS has on bacterial adhesion, this is probably due to heparin's negative charge which binds to the positively charged surface.

It was beyond the scope of this study to investigate the effect of the protein conditioning film that forms on the surface of biomaterials almost immediately following implantation of a device [44–47], however we recognise that this is an important issue that will affect subsequent biological responses including bacterial adhesion and colonisation. Bruinsma *et al*. [30] found that the adhesion of tear film proteins affected the hydrophobicity of contact lenses and subsequently affected bacteria adhesion; Lundberg *et al*. [39] found that intraocular lenses pre-incubated in cerebrospinal fluid (CSF) and plasma for 1 hour showed little difference in bacterial adhesion between the heparinised samples and non-heparinised samples, however on samples that were pre-incubated in CSF and plasma for 12 h a significantly lower number of bacteria adhered to the heparinised samples. It is suggested therefore that further *in vitro* studies looking at the affect of conditioning proteins on bacterial adhesion as well as *in vivo* investigations are necessary in order to evaluate the clinical importance of these novel heparin-complexed biomaterials.

Conclusion

The results from this study show that cationic charge significantly affects bacterial adhesion. In most cases, an increase in cationic charge corresponded with an increase in bacterial adhesion. Although an increase in bacterial adhesion was observed with increasing cationic charge, this relationship was not linear indicating that other factors are involved in bacterial adhesion. Furthermore, adhesion patterns varied between bacteria suggesting specificities between bacterial types for adhesion. The addition of heparin to these cationic MPC coatings could considerably improve many medical devices having the dual role of reducing bacterial adhesion (consequently reducing the likelihood of infection) as well as improving haemocompatibility of the device. The results also suggest that the reduction affect of heparin on bacterial adhesion to the cationic MPC copolymer coatings is stable and potentially long-term.

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