Investigation into the mechanism of bacterial adhesion to hydrogel-coated surfaces

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As a model for hydrogel-coated biomaterials, self-assembled monolayers of polyoxyethylene (POE) derivatives on sheets of polymeric biomaterials were prepared. The POE derivatives consisted of hydrophilic chains with different lengths and a long-chain alkyl group that served as an anchor function. The coatings obtained were analyzed with XPS and contact angle measurements showing hydrophilic chains of different lengths extending away from the surface. Bacterial adhesion was measured with a clinically relevant *Klebsiella pneumoniae* type strain and measurements reproduced 12 times. Bacterial adhesion decreased markedly with increasing hydrophilic chain length. Based upon these findings a new model for bacterial adhesion to hydrogel-coated surfaces is suggested: steric repulsion effects that increase with increasing chain length of grafted hydrophilic chains play an important role in bacterial adhesion to hydrogel-coated surfaces.
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1. Introduction

Medical device infections are a problem in hospitals worldwide [1]. They are caused by biofilms that develop in three stages: free surface, primary adhesion of bacteria and colonization [2]. Our aim is to stop infections by reducing the bacteria's primary adhesion to biomaterials as the first step in the cascade. One way to achieve this reduction of bacterial adhesion is the grafting of hydrogel chains to the surface of biomaterials.

In order to optimize the coatings more efficiently than by trial and error a detailed insight into the mechanism of bacterial adhesion to hydrogel-coated surfaces is necessary. So far, no completely satisfactory theory exists that can describe the interaction between a surface and bacteria [3].

In this paper, we present evidence that steric repulsion has a major influence in the reduction of bacterial adhesion to hydrogel-coated surfaces, and suggest a new model for bacterial adhesion to such surfaces that is consistent with these findings.

2. Materials and methods

2.1. Coating of polymer sheets

Polymer sheets used were standard reference polypropylene of the European Union [4] and polyamide 12 without any additives of Creanova, Marl.

Polyoxyethylene-2-cetylether (P 3769), polyoxyethylene-10-cetylether (P 5759), polyoxyethylene-40-stearate (P 3440) and polyoxyethylene-100-stearate (P 3690) were purchased from Sigma and used as received.

The amphiphiles with 40 and 100 ethylene-oxide moieties respectively per molecule, were dissolved at $c = 1\,\mathrm{mg\,l^{-1}}$ in water. The molecules with shorter hydrophilic chain lengths were dissolved at the same concentration in 50% v/v ethanol in water.

Self-assembled monolayers of the amphiphiles (see Fig. 1) were obtained by immersion of the polymer sheets in the amphiphile-solutions for 16 h at room temperature without stirring. Afterwards the coated sheets were washed by carefully dipping three times into the pure solvent and dried for 30 min at 60 °C.

2.2. Material characterization

Coated and uncoated polymer sheets underwent surface characterization in order to validate the above-mentioned surface structure.

2.2.1. X-ray photoelectron spectroscopy (XPS)

XPS spectra were taken in the surface analysis laboratory of Huels-Infracor GmbH, Marl, using a vacuum generator ESCA-LAB MK II apparatus with concentric hemispheric analyzer and two anodes. For low-resolution spectra the $\mathrm{AlK_{\alpha}}$-line at 1486.6 eV was used. High-resolution spectra were measured using the $\mathrm{MgK_{\alpha}}$-radiation at 1253.6 eV. Anodic power uptake was 300 W in both cases. In order to eliminate artefacts during the measurement quantification was performed at the beginning and end of each measurement period.

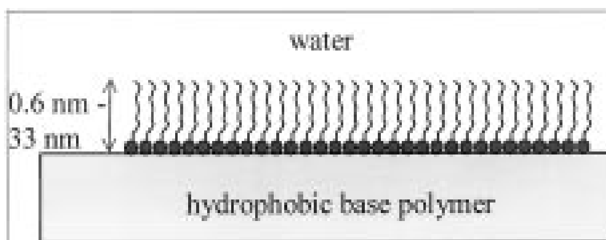

Figure 1 Self-assembled monolayer of PEO-containing amphiphiles on sheets of polymeric biomaterials. The hydrophilic chains extend away from the surface. Lengths of the chains were estimated as 0.3 nm per EO moeity [5].

2.2.2. Contact angle measurement

The contact angles of the samples were measured with sessile drops of water and methane-diiodide with a G2 computer image analysis goniometer obtained from Kruess, Germany. Advancing angles were measured at five spots on the samples and the surface energy was calculated by applying the model of Wu using harmonic means [6].

2.2.3. Bacterial adhesion measurement

Bacterial adhesion was measured indirectly by bringing the material into contact with a bacterial suspension, gentle washing and extraction of the ATP from adherent bacteria via the modification of a method first described elsewhere [7] and ATP-quantification using a bioluminescence assay.

Klebsiella pneumoniae (DSM 789) was obtained from Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany. An overnight culture of this strain was prepared by incubation in yeast–peptone–glucose (YPG) culture broth for 18 h at 37 °C. The suspension of bacteria obtained was washed three times by centrifugation at 3000 ***g*** for 15 min and resuspending the bacteria in phosphate-buffered saline (PBS).

Two hundred microliters of the bacterial suspension obtained was pipetted onto a $2 \times 2\,cm^2$ sample of the polymer sheets. After 2 h at room temperature the suspension was removed, non-adhering bacteria were washed away with PBS and ATP from adhering bacteria and was extracted by immersing the samples for 1 min at 100 °C in 10 ml of 0.1 M Tris buffer containing 4 MM ethylenediamine tetra-acetic acid (EDTA). The ATP-containing extract was frozen at -20 °C until measurement with the reagent kit CLS II from Boehringer Mannheim, Germany, with a Lumat LB 9501 obtained from Berthold, Germany.

With every coated sample an uncoated sample of the same material was measured and relative bacterial adhesion was calculated as light units measured in the case of the coated samples divided by the values of the uncoated samples times 100%.

Bacterial adhesion measurements were reproduced 12 times and the 95% confidence interval was calculated [8] and depicted as error bars in the results section.

3. Results and discussion

3.1. XPS

Surface elemental analysis via XPS has an information depth of about 10 nm and shows increasing surface oxygen contents with increasing lengths of the ethylene-oxide moieties in the molecules used for the coating procedure (see Fig. 2). In the case of polypropylene as base material the carbon content decreases at the same time.

In the case of polyamide 12 as substrate material nitrogen can be used as a marker atom for the coating process as it is found in the polymer sheet but not in the amphiphiles used for coating. The decrease of nitrogen with increasing EO-chain length is obvious in Fig. 3 and in the case of polyethylene-oxide-100-stearate the N-signal vanishes completely.

These XPS results are consistent with the oriented monolayer-structure shown in Fig. 1.

3.2. Contact angle measurements

The surface free energy of coated samples of both base materials is increased with respect to the uncoated reference (Fig. 4). Contact angle measurements have an information depth of only a few atomic layers. As EO-chain lengths higher than 10 EO-units do not yield a higher surface free energy this is further proof of the orientation of the monolayers deposited onto the base polymers.

3.3. Bacterial adhesion

Bacterial adhesion is reduced sharply with increasing hydrophilic chain lengths of the coatings applied in the case of both base polymers (Figs 5 and 6).

The bacterial adhesion values of coatings with the highest chain lengths are particularly small, although the surface free energy (Fig. 4) reaches its maximum at a hydrophilic chain length of 10 EO residues.

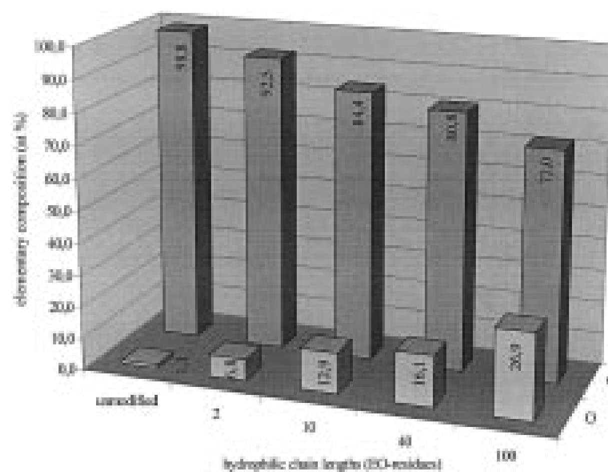

Figure 2 XPS results for polypropylene as a function of chain lengths of the hydrophilic parts of the adsorbed monolayers.

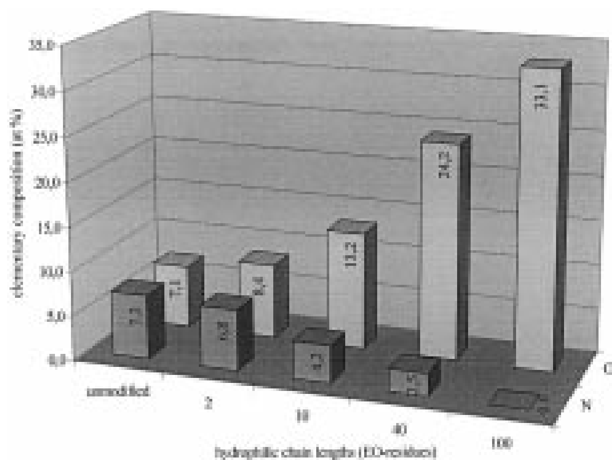

Figure 3 XPS results for polyamide 12 as a function of chain lengths of the hydrophilic parts of the adsorbed monolayers.

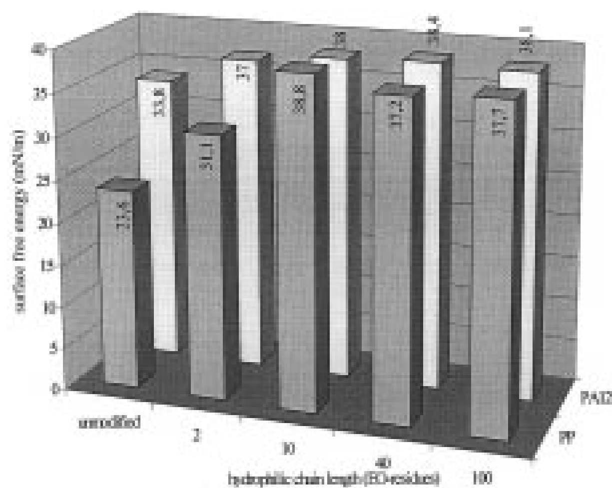

Figure 4 Surface free energy as a function of chain lengths of the hydrophilic parts of the adsorbed monolayers on poly-propylene (PP) and polyamide 12 (PA12).

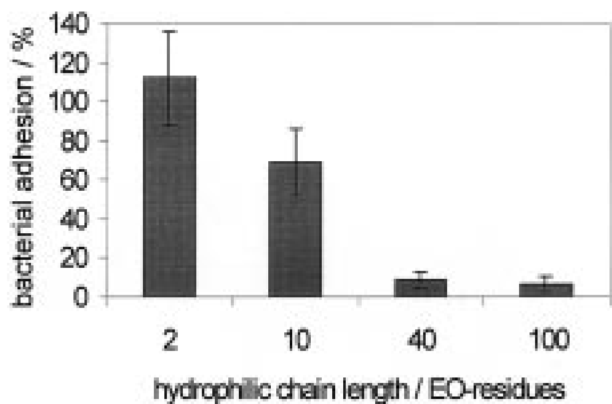

Figure 5 Relative bacterial adhesion for polypropylene as a function of chain lengths of the hydrophilic parts of the adsorbed monolayers ($n = 12,\ P = 0.95$).

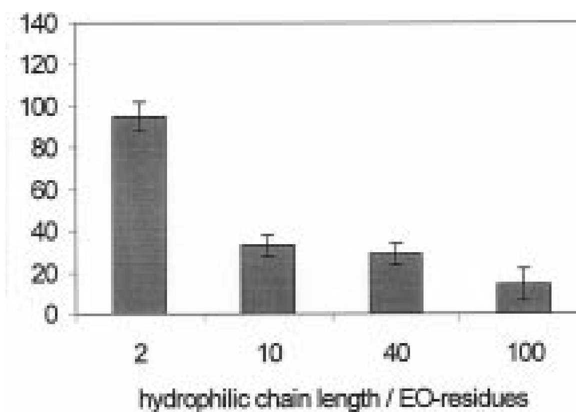

Figure 6 Relative bacterial adhesion for polypropylene as a function of chain lengths of the hydrophilic parts of the adsorbed monolayers ($n = 12,\ P = 0.95$).

Figure 7 In order to adhere to a hydrogel grafted surface a bacterium must approach the surface and reduce its distance from D to d. At this reduced distance the hydrophilic chains on both the bacterium and the coated surface are compressed, which is the cause for a repulsive steric force.

4. Conclusions

Bacterial adhesion to hydrogel-grafted surfaces decreases with increasing length of the hydrophilic chains grafted to the surface. The surface free energy of such surfaces does not vary significantly with the length of the chains above 10 EO units. Therefore, the surface free energy approach used frequently in the literature [3] cannot explain the bacterial adhesion differences in the case of hydrogel-coated surfaces.

To our knowledge these data are the first experimental proof for the assumption that steric interactions play a major role in bacterial adhesion to hydrogel surfaces. Such steric interactions can be described by the Alexander–de Gennes Equation [9]

$$\Delta G_{\text{ster}} = \frac{kT}{\pi}\frac{L}{s^3}\left[\left(\frac{2L}{D}\right)^{\frac{9}{4}} - \left(\frac{D}{2L}\right)^{\frac{3}{4}}\right]$$

The repulsive energy ΔG_{ster} between a particle approaching a hydrogel-grafted surface to a distance D increases with the chain length L of the grafted hydrogel chains. It is also dependent upon the graft density $1/s^2$.

The mechanism of bacterial adhesion to hydrogel grafted surfaces can therefore be viewed as shown in Fig. 7.

The reduced bacterial adhesion to hydrogel-grafted surfaces is caused by steric repulsion effects between the teichonic acid (Gram-positive) or lipopolysaccharide (Gram-negative) hydrogels on the bacterial surfaces [10] and the hydrogel chains on the coated materials.

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Received and accepted 17 February 1999