



Grafted thermo- and pH responsive co-polymers: Surface-properties and bacterial adsorption

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Abstract

A series of responsive polymers displaying pH and temperature-mediated phase changes were prepared from *N*-isopropylacrylamide and ω -carboxylic acid functionalised acrylamides. These polymers were grafted to surfaces and their characteristics probed by atomic force microscopy in aqueous solutions. The effects of pH and temperature induced phase transitions on the short-term adsorption of the bacteria *Salmonella typhimurium* and *Bacillus cereus* from pure cultures were assessed. Contact angle studies indicated that pH and temperature-dependent surface properties were exhibited by the graft polymer surfaces. Temperature-dependent surface morphology changes occurred through polymer graft phase transitions as observed in AFM and accompanying changes in adhesion forces underwater were found to correlate with surface properties obtained from contact angle measurements. Adsorption of *S. typhimurium* and *B. cereus* was not significantly altered as a function of pH, but attachment of both bacterial strains increased at temperatures above the polymer coil-globule transition indicating the importance of switching surface hydrophobicity in controlling short-term bacterial adsorption.

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1. Introduction

The possibility of generating synthetic polymers that can control bioadhesive processes such as cell attachment is of considerable industrial significance and clinical importance, as well as being a topic of much

scientific interest (Blackwood, 2003). Potential applications for synthetic materials that are either bioadhesive or biocompatible are extremely widespread, ranging from surgical implants (Fournier et al., 2003; Hron, 2003), supports for in vitro cell culture and biotechnological screening (Hersel et al., 2003; Noiset et al., 2000; Neff et al., 1999), scaffolds for tissue engineering (Shin et al., 2003; Vats et al., 2003; Zacchi et al., 1998) through to anti-fouling surfaces or coatings in industrial processing and marine environments (Pasmore

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et al., 2002; Taton and Guire, 2002; Lewis et al., 2001; Brady, 1997).

In recent years, there has been a focus on preparing 'active' or responsive materials that exhibit different properties, such as a phase transition, under differing environmental conditions in order to manipulate or control bioadhesive interactions (Kazanci, 2003; Kwon et al., 2003; Yamato et al., 2002, 2000; Malmstadt et al., 2003; Lackey et al., 2002; Hoffman, 2000; Galaev and Mattiasson, 1999; Inoue et al., 1997). Potential uses for these classes of polymers include targeted delivery of drugs (Doorty et al., 2003) and anti-microbials or as 'smart' surfaces that can be switched from an adhesive to a non-adhesive state (Ebara et al., 2003; Rao et al., 2002). One area of particular interest is the utilisation of responsive polymers to control the attachment of prokaryotic cells to surfaces. Pioneering work by Lopez and co-workers demonstrated that attachment of *Halomonas* and *Staphylococcus* species to substrates could be controlled by surface-grafted poly(*N*-isopropylacrylamide) (PNIPAm), with greater numbers of cells adsorbing to the surface when it was of a wettability favourable for the individual bacterial species (Ista and Lopez, 1998; Ista et al., 1999). Further work by this group established that more complex organisms were able to detect substrates of different wettabilities, indicating the importance of surface physical chemistry on bioadhesion (Finlay et al., 2002).

We have been investigating whether the hydrophilic–hydrophobic switch of PNIPAm co-polymers at their lower critical solution temperature (LCST) can mediate the attachment of common pathogens and have reported the effects of neutral PNIPAm co-polymer switching on the adsorption of proteins as model conditioning films and on the attachment and release of representative microorganisms *Listeria monocytogenes*, *Salmonella typhimurium* and *Bacillus cereus*. Bioadhesion to these polymer grafts and brushes was found to be dependent on LCST-mediated changes in surface physico-chemistry and correlations were obtained between polymer conformations as observed in AFM and contact angle goniometry and overall extent of protein and cell attachment (Cunliffe et al., 2003, 2000).

However, the fact that most bacteria are negatively charged at ambient pH suggested that further control over bacterial adsorption might be achieved by utilising PNIPAm co-polymers containing anionic or acidic

co-monomers as the surface-displayed brushes. We chose *S. typhimurium* and *B. cereus* as the test bacteria for these assays to afford a direct comparison with our earlier work and because these microorganisms are implicated in a variety of medical conditions (McCabe-Sellers and Beattie, 2004; Monack et al., 2004). In this paper, we report the first investigation into the effect of temperature and pH-mediated phase transition of these polymers on the short-term adsorption of *S. typhimurium* and *B. cereus*.

2. Materials and methods

High purity reagents, monomers and solvents for chemical synthesis were purchased from Aldrich, Acros or Fisher Scientific (UK) and used as received. Inhibitors were removed from *N*-isopropylacrylamide (NIPAm) and *N*-*t*-butylacrylamide by recrystallisation from hexane.

For contact angle goniometry, double distilled water (DDW, surface tension 72.8 mN m^{-1} at 20.0°C) or phosphate buffered saline (150 mM NaCl at pH 5.6 or 7.4) were used. For these measurements a Kruss G10 contact angle measuring system equipped with a sealed humidity-controlled sample chamber, and automated image analysis system was used. Drops of liquid of known volume (1–4 μL) were applied from a micro-syringe to the surface of the polymer graft surfaces through a small port at the top of the cell: to avoid cross-contamination of liquids, a dedicated micro-syringe was used for each diagnostic liquid. Measurements of contact angles was conducted with a precision of $\pm 0.5^\circ$.

2.1. Monomer and polymer synthesis

6-Acryloylamino-hexanoic acid (6-AHA) was prepared by the method of Kuckling et al. (2000) and recrystallised before use: analytical data was consistent with the desired product.

Polymer P1: *N*-Isopropylacrylamide (4.52 g, 40 mmol) and *N*-*t*-butylacrylamide (1.27 g, 10 mmol) were dissolved in propan-2-ol (40 mL) in a thick walled Schlenk tube, and 3-mercaptopropanoic acid (0.018 g, 0.172 mmol) and 4,4'-azobis(4-cyanovaleric acid) (0.4 g, 1.42 mmol) were added. The solution was degassed by freeze-thaw cycles under vacuum at least three times and placed in a thermostatted oil bath at

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