CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200600342

Affinity Adhesion of Carbohydrate Particles and Yeast Cells to Boronate-Containing Polymer Brushes Grafted onto Siliceous Supports

Alexander E. Ivanov,*[a] Homayon Ahmad Panahi,[b, c] Marina V. Kuzimenkova,[a] Lars Nilsson,[d] Björn Bergenståhl,[d] Husain S. Waqif,[c] Mohsen Jahanshahi,[e] Igor Yu. Galaev,[a] and Bo Mattiasson[a]

Abstract: Cross-linked agarose particles (Sepharose CL-6B) and baker's yeast cells were found to adhere to siliceous supports end-grafted with boronate-containing copolymers (BCCs) of *N*,*N*-dimethylacrylamide at pH $\geq$ 7.5, due to boronate interactions with surface carbohydrates of the particles and the cells. These interactions were registered both on macroscopic and on molecular levels: the BCCs spontaneously adsorbed on the agarose gel at pH $\geq$ 7.5, with adsorption increasing with pH. Agarose particles and yeast cells stained with Procion Red HE-3B formed stable, monolayer-like structures at pH 8.0, whereas at pH 7.0–7.8 the structures on the copolymer-grafted supports were less stable and more random. At pH 9.0, 50 % saturation of the surface with adhering cells was attained in 2 min. Stained cells formed denser and more stable layers on the copolymer-grafted supports than they did on supports modified with self-assembled organosilane layers derivatized with low-molecular-weight boronate, presumably due to a higher reactivity of the grafted BCCs. Quantitative detachment of adhered particles and cells could be achieved by addition of 20 mM fructose—a strong competitor for binding to boronates—at pH 7.0–9.0. Regeneration of the grafted supports allowed several sequential adhesion and detachment cycles with stained yeast cells. Affinity adhesion of micron-sized carbohydrate particles to boronate-containing polymer brushes fixed on solid supports is discussed as a possible model system suggesting a new approach to isolation and separation of living cells.

Keywords: boronic acid · cell adhesion · chemisorption · molecular recognition · oligosaccharides

Introduction

Polymer brushes grafted onto solid supports have been reported to create favorable microenvironments for the capture of microbial cells from contacting aqueous media.[1–3] The charged functional groups (such as diethylaminoalkyl groups, DEA) carried by the flexible tethered chains provide a three-dimensional network that facilitates cell interaction with and attachment to surfaces.[1,2] The rate constant for capture registered with DEA brushes attached to polyethylene fibers was 1000 times higher than that found with wide-pore, cross-linked poly(glycidyl methacrylate) beads modified with DEA functions.[2]

Neutral hydrophilic polymer brushes, on the other hand, have been found to prevent adhesion of bacteria[4,5] and, therefore, formation of infectious pathogenic biofilms.[3,6] Such prevention is possible because of steric hindrance exerted by the brush, which keeps the bacteria at a distance from the solid surface.[4] It appeared an interesting challenge to synthesize an affinity polymer brush capable of a transition from an adsorbing to a nonadsorbing state as a re-

[a] Dr. A. E. Ivanov, M. V. Kuzimenkova, Dr. I. Yu. Galaev, Prof. B. Mattiasson
Department of Biotechnology
Center for Chemistry and Chemical Engineering
Lund University, P.O. Box 124, 22100, Lund (Sweden)
Fax: (+46) 46-222-4713
E-mail: alexander.ivanov@biotek.lu.se

[b] H. A. Panahi
Department of Chemistry, Central Teheran Campus
Islamic Azad University, Tehran (Iran)

[c] H. A. Panahi, Prof. H. S. Waqif
Department of Chemistry, Science and Research Campus
Islamic Azad University, Tehran (Iran)

[d] L. Nilsson, Prof. B. Bergenståhl
Department of Food Technology
Center for Chemistry and Chemical Engineering
Lund University, P.O. Box 124, 22100, Lund (Sweden)

[e] Prof. M. Jahanshahi
Department of Chemical Engineering, Faculty of Engineering
The University of Mazandaran, Babol (Iran)

sponse to some small environmental change, such as a change in pH or buffer composition. Brushes of this kind would be promising for controlled uptake and release of cells or for separation of different cell types by specific affinity interactions.

Incomplete detachment of cells adhered to solid matrices, however, is a common problem in the development of techniques for cell separations based on the above interactions.[7,8] Recovery of both microbial and mammalian adhered cells depends on the method of elution,[7] as well as on the ligand density and bond strength between the cells and affinity ligands.[8] In the detachment step, polymer brushes may have some advantages over ligands fixed on cross-linked rigid supports as affinity adsorbents for cells. First, a polymer brush may undergo cooperative transitions between the swollen and the collapsed states of its chains,[9] which can affect the adhesion of mammalian[10] or bacterial cells[11] even in the absence of any specific ligands (that is, solely through temperature-triggered changes in the hydrophobicity and structure of the brush). Second, a polymer brush carrying interactive ligand groups might dissociate more easily from the cell wall because of a gain in the conformational entropy of the grafted chains, with the entropy decreasing with penetration of a particle into a brush[12] and increasing when the chains return to an unbound and flexible state.

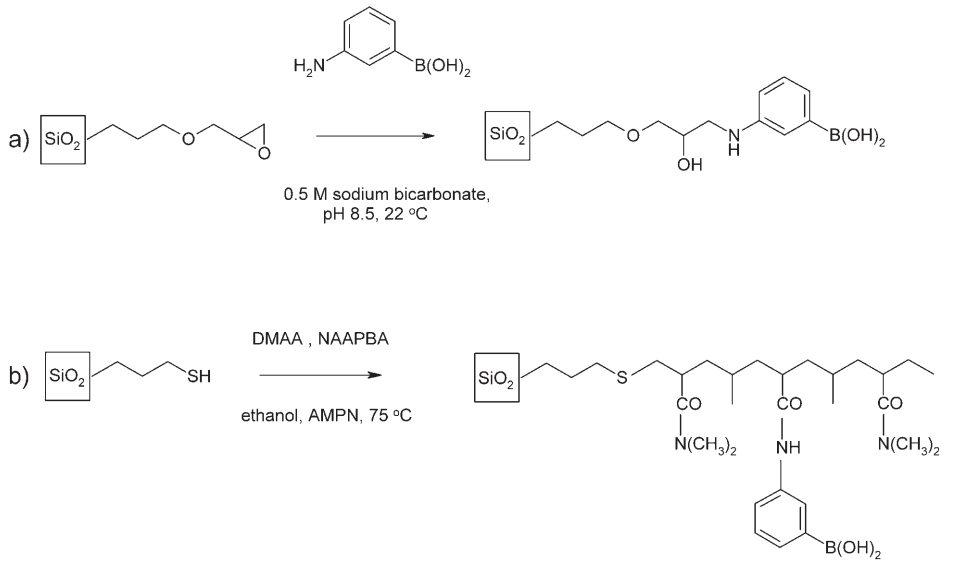
One could expect that small ligand groups of relatively weak affinity (K_{ass} = 10–10^3 M^{-1}) and with high association rates should provide a more reversible type of interaction with cell walls than high-affinity ligands such as immobilized cell-adhesive peptides or proteins[13] ($K_{ass} \approx 10^6$–10^7 M^{-1}[14]). Weak binding of mono- or oligosaccharides to immobilized antibodies or lectins is typically characterized by K_{ass} values of 10^2–10^4 M^{-1}.[15] Chromatographic separations of saccharides on immobilized protein columns have generally been performed in isocratic mode, due to the high association and dissociation rate constants, resulting in separation of the saccharides in short times.[15,16] These facile reversible interactions are probably one of the reasons why sugar-specific proteins have been successfully used for cell attachment and detachment procedures,[7] as well as for isolation of populations of living cells.[17]

Another important reason for the use of sugar-specific antibodies and lectins for the controlled attachment and detachment of cells is the wide variety of poly- and oligosaccharides localized on the walls of microbial[18] and animal[19] cells, which can be targeted by sugar-specific ligands. One can conceive of a polymer brush populated with many such ligands interacting with the cell saccharides in a selective way, so that the differences in cell wall carbohydrate composition and structure could be reflected in the binding process. A well known small sugar-specific ligand is phenylboronic acid (PBA).[20] Water-soluble synthetic polymers containing PBA have been found to interact with red blood cells and to modulate their immunological properties,[21] to induce proliferation of lymphocytes,[22] and to inhibit leukocyte adhesion to monolayers of endothelial cells.[23] These findings suggest potential for cell adhesion to polymer brushes containing PBA as a pendant ligand group.

The aim of this study was to find out whether relatively large carbohydrate-containing particles, such as cross-linked agarose gel particles (80–160 μm) or stained yeast cells (4–6 μm), could adhere to solid surfaces grafted with boronate-containing copolymers (BCCs) and be detached from the surfaces on demand. Such potential might suggest a new approach to isolation and separation of living cells. The aim of this paper was to compare the adhesivity of such boronate-containing polymer brushes with that of low-molecular-weight boronates immobilized on the same solid supports, to find conditions for particle detachment from the polymer-grafted surfaces, and to evaluate the performances of these surfaces during repeated particle attachment and detachment procedures.

Results and Discussion

Surface modification and characterization of glass plates and capillary tubes: Glass plates and capillary tubes were chemically modified with self-assembled layers of organosilanes containing glycidyl or mercapto groups. The glycidyl groups were used for direct immobilization of 3-aminophenylboronic acid by the known technique[24] (see Scheme 1a),



Scheme 1. *m*-Aminophenylboronic acid immobilization on 3-glycidoxypropylsilylated supports (a) and grafting of the phenylboronate-containing copolymer to 3-mercaptopropylsilylated supports (b).

whereas the mercaptopropylsilylated supports were grafted with the copolymer of *N*,*N*-dimethylacrylamide (DMAA) and *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA; see Scheme 1b). A new method for the preparation of an end-tethered monolayer of poly(*N*-isopropylacrylamide) has recently been developed: chemical attachment of the polymer to the resistive heater lines of silicon chips was achieved by radical polymerization of the monomer accompanied by grafting of the polymer to mercaptoalkyl groups of the organosilane immobilized on the carrier.[25] This type of polymer grafting has been shown to result in 15–30-fold reductions in protein adsorption on silica gel.[26] In this study we used a similar grafting technique for chemical modification of plane glass surfaces with DMAA-NAAPBA copolymer (see Experimental Section).

Chemical modification and polymer grafting of the siliceous supports were confirmed by the relevant changes in wettability of the carriers. Glass plates and capillary tubes cleaned with sodium hydroxide and hydrochloric acid (see Experimental Section) were almost fully wettable with water, whilst the modification of glass supports with 3-mercaptopropyltrimethoxysilane (3-MPTMS) or 3-glycidoxypropyltriethoxysilane (3-GPTMS) resulted in more hydrophobic surfaces. The water contact angles of the modified glass capillaries and plates are listed in Table 1. The water contact

Table 1. Water contact angles of chemically modified glass plates and capillaries at 25 °C.

Type of chemical modification	Water contact angle [°]
3-MPTMS-glass	67 ± 4;[a] 62 ± 1[b]
3-MPTMS-glass grafted with DMAA-NAAPBA copolymer	35 ± 5;[a] 49 ± 4[b]
3-GPTMS-glass	
nonhydrolyzed	54 ± 4
epoxy groups hydrolyzed to diols[a]	40 ± 3
3-GPTMS-glass with immobilized 3-APBA[a]	45 ± 4

[a] Estimated by capillary rise. [b] Estimated by direct drop shape analysis.

angle of MPTMS glass at 25 °C was found to be $62°\pm1°$ by direct drop shape analysis and $67°\pm4°$ by measurements of capillary rise. Water contact angles measured at room temperature on glass plate and silicon chip surfaces chemically modified with 3-MPTMS in anhydrous cyclohexane were recently reported to be $69°\pm2°$.[27]

Subsequent grafting of the copolymer of *N*,*N*-dimethylacrylamide and *N*-acryloyl-*m*-aminophenylboronic acid (DMAA-NAAPBA copolymer) to 3-MPTMS-modified glass resulted in a decrease in its water contact angle from 62° to 49° (see Table 1), indicating decreased hydrophobicity of the surface caused by the grafting of a hydrophilic copolymer. In fact, DMAA-NAAPBA copolymer containing 9 mol % NAAPBA is soluble in water at concentrations up to 24 mg mL^{-1} over a wide pH range.[28]

Strong evidence of successful grafting of the BCC was provided by the time dependences of the contact angles of pure water, of 20 mM sodium bicarbonate buffer (pH 9.0), and of 100 mM fructose solution in the same buffer on the grafted surface (see Figure 1). As follows from Figure 1, the most stable contact angle value was that exhibited by pure

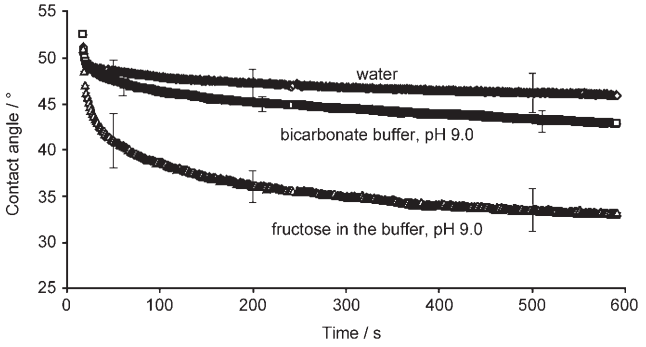


Figure 1. Contact angles of water (◇), of sodium bicarbonate buffer (20 mM, pH 9.0; □), and of fructose solution in the same buffer (100 mM; △) on the copolymer-grafted glass plates as functions of time at 18 °C. The error bars designate the standard deviation obtained in three independent experiments.

water after mechanical equilibrium had been obtained and the surface had been hydrated (after ca. 30 s). The area occupied by the droplet remained almost constant at contact times longer than 200 s (see Figure 2). In the case of 20 mM

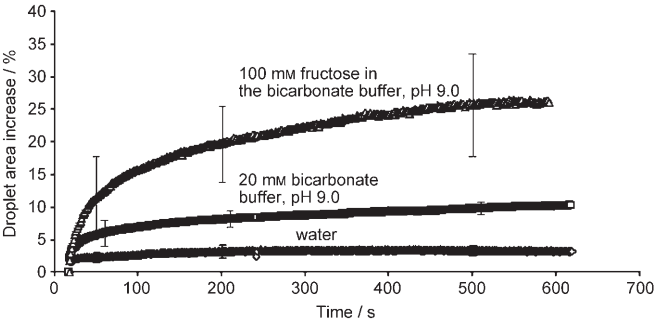


Figure 2. Areas occupied by droplets of water (◇), of sodium bicarbonate buffer (20 mM, pH 9.0; □), and of fructose solution in the same buffer (100 mM; △) on the copolymer-grafted glass plates as functions of time at 18 °C. The error bars designate the standard deviation obtained in three independent experiments.

sodium bicarbonate buffer (pH 9.0) a gradual decrease in the contact angle due to droplet spreading could be seen. This spreading can be explained in terms of ionization of the PBA groups of the copolymer at the above pH,[29] endowing the grafted surface with better wettability. The presence of 0.1 M fructose in the bicarbonate buffer resulted in even stronger changes in the contact angle and the droplet area with time, however (see Figure 1 and Figure 2), with the contact angle gradually decreasing down to 31°–35° over about 550 s. The experiment was repeated three times on fresh surfaces: the results were somewhat scattered (indicated by the error bars in Figure 1), but the obtained contact

angles were always smaller than those observed just in the presence of the buffer.

Among many sugars, fructose forms the most stable complexes with BCCs.[29] Complex formation between the sugar and the grafted copolymer resulted in increasing hydrophilicity of the polymer layer, as reflected in the time dependences of the droplet area and the contact angle. In general, low-molecular-weight hydrophilic solutes, such as sugars, influence neither the surface tension of water nor the contact angle between water and surfaces: fructose, for instance, had almost no effect on the wettability of a 3-mercaptopropyl-modified glass surface (see Figure 3). The obvious effect

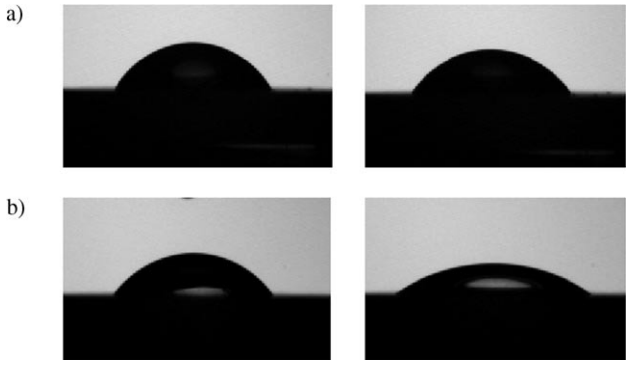


Figure 3. Photographs of droplets (4 μL) formed by bicarbonate buffer (20 mM, pH 9.0) containing fructose (100 mM) on 3-mercaptopropyl-modified glasses (a) and on the glasses grafted with DMAA-NAAPBA copolymer (b), taken 25 s (left) and 500 s (right) after droplet deposition.

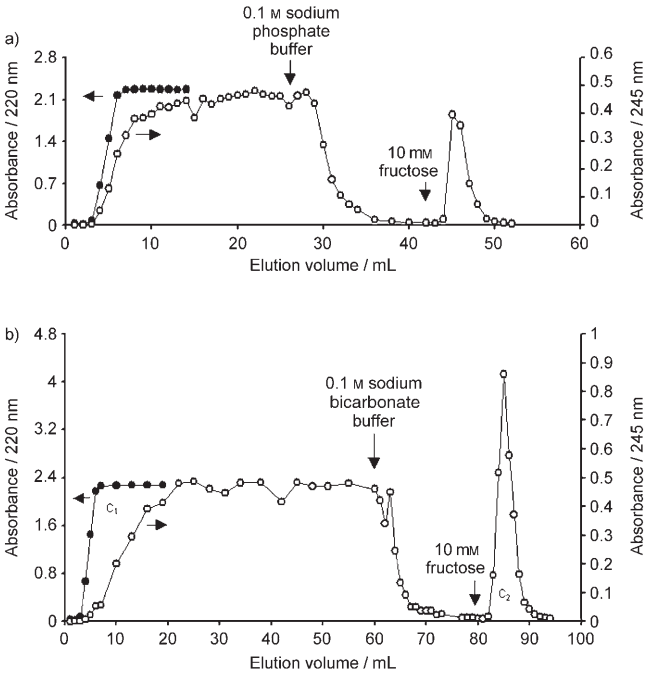


Figure 4. Frontal chromatogram of DMAA-NAAPBA copolymer (○) and poly(*N*,*N*-dimethylacrylamide) (●) on a Sepharose CL-6B column (1 × 2.1 cm) in: a) sodium phosphate buffer (0.1 M, pH 7.9, 22 °C), and b) sodium bicarbonate buffer (0.1 M, pH 9.2, 22 °C). The absorbance in the fractions was measured after 100-fold dilution with buffer at 220 nm and 245 nm for poly(*N*,*N*-dimethylacrylamide) and DMAA-NAAPBA copolymer, respectively. Arrows indicate application of fructose (10 mM) in the above buffer solutions.

observed on the BCC-grafted surface is clear evidence of its specific interaction with the dissolved fructose, and so the sequential wettability experiments clearly support the scheme of chemical grafting suggested in Scheme 1.

The water contact angles for 3-GPTMS-modified glass were $40 \pm 3°$ and $54 \pm 2°$ for the samples with hydrolyzed and nonhydrolyzed epoxy functions, the latter being comparable to the data found in the literature for the similar modification of glass surfaces (51°).[30]

Interaction between DMAA-NAAPBA copolymer and agarose gel: It was found that the DMAA-NAAPBA copolymer could be strongly adsorbed by Sepharose CL-6B (a neutral, cross-linked agarose gel) from aqueous solutions with pH > 7.0, unlike the homopolymer of *N*,*N*-dimethylacrylamide, which eluted close to the total volume of the column (Figure 4). This indicated binding of pendant PBA groups to the polysaccharide gel. The adsorption capacity of the gel could be calculated either from the difference between the two normalized breakthrough curves (C_1; see Figure 4b) or from the summed up absorbance of elution peaks registered at $\lambda_{max} = 245$ nm (C_2). C_1 and C_2 exhibited fairly high and similar values of 18 and 15 mg mL^{-1} gel, respectively, which indicated low desorption rates and essential irreversibility of the copolymer adsorption from the equilibration buffer. The low desorption rates are basically typical of proteins exhibiting high equilibrium association constants ($> 10^4$ M^{-1}) with immobilized affinity ligands[31] and suggest a multivalent character of the copolymer adsorption: that is, its interaction with the gel through several PBA groups. It is worthwhile to note that adsorption of monomeric NAAPBA to Sepharose CL-6B is completely reversible and characterized by slight retardation and a chromatographic capacity factor of 1.8 at pH 9.2 (data not shown). Elution of DMAA-NAAPBA copolymer from Sepharose CL-6B was achieved by application of fructose, a strong competitor for sugar binding to boronate: the association constant of fructose with borate ($\log K_{ass} = 3.2$) is one of the highest among the monosaccharides.[32] The fructose-assisted elution demonstrates the specific character of copolymer adsorption based on boronate–sugar interactions.

Agarose is mainly composed of alternating D-galactose and 3,6-anhydro-L-galactose units, with the galactose units bound to the neighboring units through the 1- and 3-hydroxy groups.[33] The hydroxy groups in the 4- and 6-positions of the galactose units may, in principle, be accessible for binding to NAAPBA. Interaction between borate ion and a galactose 4,6-diol motif had earlier been demonstrated by ^{11}B and ^{13}C NMR spectroscopy, although the value of the association constant is probably lower than that of free D-galactose ($\log K_{ass} = 2.10$)[32] because it is the glycosidic hydroxy group of galactose that plays a major role in the interaction of the sugar with borate.[34] One cannot exclude participation of agarose chain end groups and glycerol moieties

of the hydrolyzed dibromopropane cross-linker[35] in the complex formation with boronates. All these interactions are, however, weaker than the boronate interaction with fructose used for the competitive elution of DMAA-NAAPBA copolymer.

It follows from Figure 4 and Table 2 that the copolymer adsorption capacity decreases with decreasing pH. As it is the anionic form of PBA that is capable of complex formation with diols and sugars,[20] a lower content of reactive anions in the chains results in lowered adsorption of the copolymer. Thanks to the adsorption of the copolymer on agarose, followed by the fructose-assisted desorption, one can reasonably expect reversible adhesion of the agarose particles to a plain surface populated with end-grafted chains of DMAA-NAAPBA copolymer: that is, to a polymer brush featuring boronate affinity functions.

Table 2. Adsorption of DMAA-NAAPBA copolymer on Sepharose CL-6B at different pH values.[a]

pH	DMAA-NAAPBA [mg mL^{-1} gel]
9.2	15
8.3	10
7.9	6
7.5	2
7.1	0

[a] Copolymer adsorption (C_2) was calculated from the UV absorbance in the eluted peaks estimated at 245 nm; see Experimental Section.

Adhesion of agarose particles to the DMAA-NAAPBA-grafted glasses: The grafted glass plates were exposed to a sedimenting suspension of Sepharose CL-6B in 0.1 M sodium bicarbonate buffer (pH 9.2) and rinsed with the buffer, and the plates were tested for turbidity at 500 nm. Figure 5 shows the development of the turbidity over time. Once some weakly bound particles had spontaneously detached from the surface, the rinsing of the adhesion layer with several portions of fresh buffer resulted in a stable layer of strongly adhered particles. These latter particles could be detached after incubation with the sodium bicarbonate buffer containing 20 mM fructose (pH 9.2). This fructose-assisted detachment of particles demonstrated that their adhesion was due to specific boronate–sugar interactions. Note that the unmodified glasses retained far fewer agarose particles (see Figure 5).

Although the turbid layer of attached agarose particles was visible to the naked eye, better visualization of the particles could be achieved by staining particles with Procion Red (see Figure 6a). On some glasses the grafted polymer layer was etched with 4 M NaOH to destroy Si–O–Si bonds[36] between the glass surface and the grafted polymer. One can observe much poorer particle population on the etched zones than in the densely populated surrounding area (see Figure 6b).

Adhesion of the stained yeast cells to the DMAA-NAAPBA-grafted glasses and to glasses containing 3-APBA immobilized through the low-molecular-weight silane (3-GPTMS): As the large carbohydrate particles of Sepharose CL-6B (80–160 μm) could be specifically adhered to and detached from the BCC-grafted surfaces, one could reasonably expect much smaller bakery yeast cells (ca. 5 μm) to behave similarly: that is, to interact with the grafts through the oligosaccharides bound to their surfaces. Yeast cells had earlier been employed as a model for investigation of cell adhesion to surfaces containing immobilized lectins and of the detachment forces needed for cell removal.[7] These cells contain large quantities of mannose, mostly situated in *N*- and *O*-glycans localized in the cell walls.[19] Mannose residues in glycans are most often connected by 1–2 or 1–6 bonds, so the 2,3- or 4,6-diol motifs of the mannose residues should therefore be available for interaction with borate. The local association constants of the 2,3- and 4,6-diol motifs of methyl α-D-mannopyranoside with borate have been reported to be 15 and 3 M^{-1}, respectively.[31]

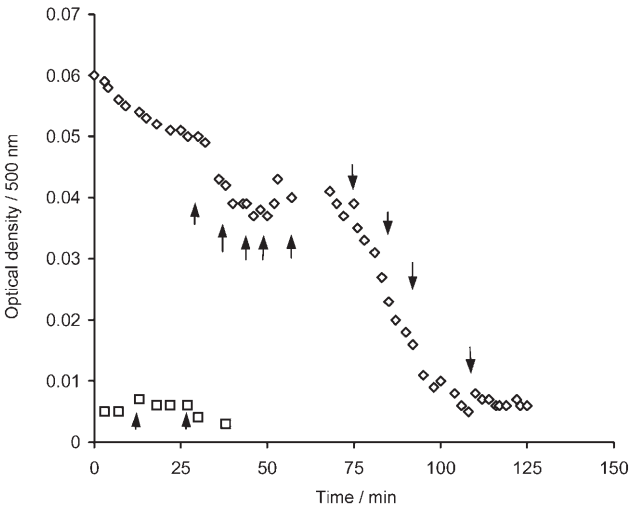


Figure 5. Spontaneous and fructose-assisted detachment of Sepharose CL-6B particles from glass plates grafted with DMAA-NAAPBA (◇) and from unmodified glass plates (□). Upward arrows indicate turning upside-down of a closed cuvette containing the glass with a fresh portion of sodium bicarbonate buffer (0.1 M, pH 9.2, 10 swings in ca. 30 s). Downward arrows indicate the same procedure in the presence of fructose (20 mM).

The yeast cells were killed by heating and stained with Procion Red dye. They withstood multiple centrifugation and resuspension procedures well (see Experimental Section) and can therefore be regarded as relatively stable, few-micron-sized particles exhibiting surface carbohydrates. Adhesion of the stained cells onto the glasses grafted with DMAA-NAAPBA produced a pattern similar to that seen with the agarose particles: the layer of the adhered cells on the etched glass plate is shown in Figure 6c. Detachment of the weakly adhered cells was followed by a slight decrease in the absorbance of the cell layer, which exhibited good stability on extensive rinsing with 0.5 M bicarbonate buffer, but quickly disintegrated after rinsing with 0.1 M fructose dissolved in the same buffer (see Figure 7), due to the competitive interaction of the sugar with PBA groups. The adhered

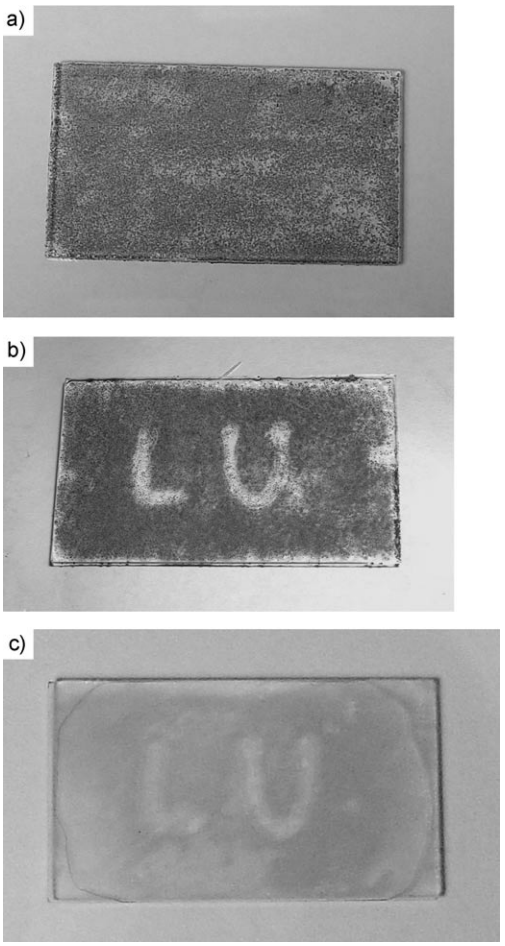


Figure 6. Photographs of the DMAA-NAAPBA-grafted glass plates (25 × 45 mm) with adhered stained Sepharose CL-6B particles (a, b) and stained yeast cells (c): a) glass with no etching, b, c) glasses with LU pattern etched by alkali.

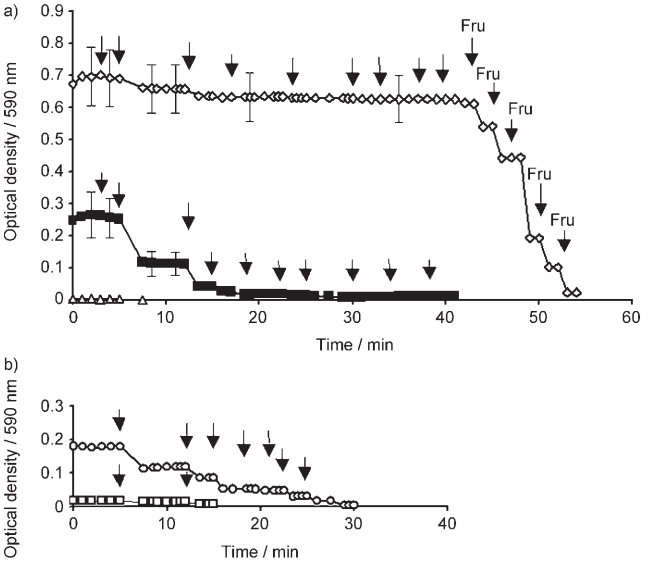


Figure 7. Spontaneous and fructose-assisted detachment of the stained yeast cells from the glass plates. a) Glass plates end-grafted with DMAA-NAAPBA (◇), chemically modified with 3-GPTMS and PBA (■), and unmodified (△). b) Glass plates chemically modified with 3-MPTMS (○) and end-grafted with polyDMAA (□). The cell suspension was allowed to precipitate for 1 h onto all types of glasses from sodium bicarbonate buffer (0.5 M, pH 9.0). Arrows indicate the addition of fresh buffer and turning upside-down of a closed cuvette (see Experimental Section). Decimolar fructose in the buffer was used for cell detachment. Six independent experiments were made with both types of PBA-containing glasses. The error bars indicate the 95% confidence range.

cells were quantitatively released into the contacting aqueous phase in about 10 min. At fructose concentrations of 20–50 mM the complete disintegration of the adhered cell layer took about 30 min, while at concentrations of 10 mM and lower the complete disintegration could not be achieved within 1 h (data not shown). Sedimentation of the stained yeast cells onto the grafted plates from buffer solutions with lower ionic strength or lower pH resulted in weaker attachment of the cells; this effect is considered below.

Obviously, the stained yeast cells displayed much higher affinity to DMAA-NAAPBA-grafted glasses than to unmodified glasses and to the glasses containing PBA immobilized through self-assembled layers of 3-GPTMS (see Figure 7). Although the last sample (PBA-GPTMS-modified glass) also exhibited a certain affinity towards the yeast cells, the stability of the cell layer was relatively low, so that only a few runs of rinsing with 0.5 M bicarbonate buffer solution (pH 9.0) resulted in the disintegration of the layer. It appears that the PBA groups located along the end-grafted flexible chains were more mobile and reactive than those immobilized close to the solid surface. To illustrate the possible effects of the MPTMS-sublayer and the polyDMAA-grafts on the cell adhesion, control experiments were carried out with the glasses modified with the above coatings. As follows from Figure 7 (lower part), the MPTMS-modified glasses exhibited some cell adhesion, but this, however, was largely neutralized by grafting of polyDMAA performed in a similar manner to the grafting of DMAA-NAAPBA copolymer. The cell adhesive properties of DMAA-NAAPBA-glasses were certainly due to the pendant PBA groups attached to the grafted polymer chains.

Effects of precipitation time, pH, and ionic strength on adhesion of stained yeast cells on DMAA-NAAPBA-grafted glasses: As follows from Figure 7, the integrity of the adhered stained cell layers was reflected in their optical density (λ_{max} = 590 nm), which was typically 0.6–0.7 after rather long contact (1 h) of the glass supports with sedimenting cell suspension, followed by extensive rinsing. In order to evaluate the kinetics of the cell attachment, DMAA-NAAPBA-grafted glasses were exposed to the sedimenting suspension for different times. Figure 8 shows the time dependence of optical density of the adhered cells. The cell attachment took place very quickly, with the optical density of the cell layer having already reached half of its saturation value in 2 min. On the other hand, the further saturation of the copolymer-grafted surface took longer, and seemed to reach a constant value after 1 h.

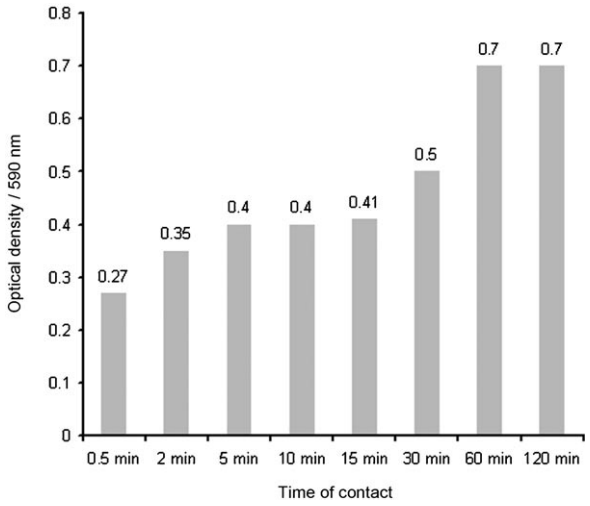


Figure 8. Optical density of the stained yeast cells adhered on the glass plates grafted with DMAA-NAAPBA copolymer as a function of contact time at pH 9.0. The glass plates were allowed to contact the sedimenting cell suspension for the given time, rinsed with a stream of sodium bicarbonate buffer solution (0.5 M, 3 mL), placed in a glass cuvette with the buffer solution, and photometered at 590 nm.

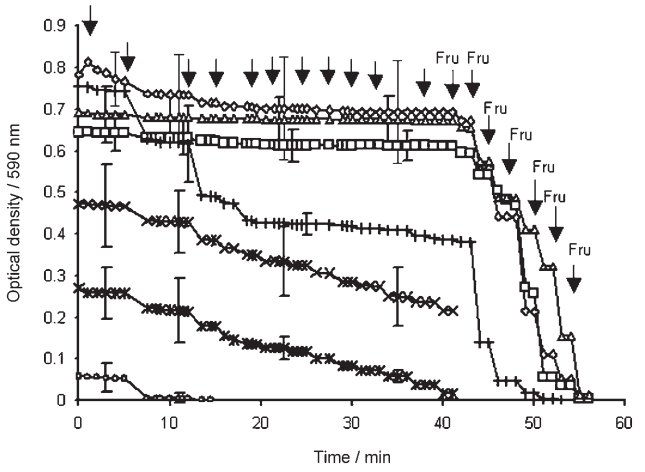


Figure 9. Spontaneous and fructose-assisted detachment of the stained yeast cells from the glass plates grafted with DMAA-NAAPBA copolymer at different pH values: 9.0 (◇), 8.5 (□), 8.0 (△, +), 7.8 (×), 7.5 (*), and 7.0 (○). Sodium bicarbonate (0.5 M, pH 8.0–9.0) and sodium phosphate (0.5 M, pH 7.0–7.8) buffer solutions were used in all cases except for the profile (+), obtained in sodium phosphate buffer (10 mM) containing NaCl (0.15 M, pH 8.0). The cell suspension was allowed to contact the glasses for 1 h at each pH. Arrows indicate the addition of a portion of fresh buffer and turning upside-down of a cuvette containing the glass. Decimolar fructose in the buffer was used for cell detachment. Three independent experiments were performed at each pH. The error bars indicate the standard deviation.

The pH dependence of complex formation between the boronate-containing polymers and the oligosaccharides of the stained yeast cells is important for capability to manipulate living cells under nondestructive conditions, preferably close to physiological ones. DMAA-NAAPBA copolymers had earlier been shown to bind to murine spleen lymphocytes at pH 7.4 and thereby to induce their proliferation.[22] Although the binding of boronate-containing copolymers (BCCs) to mono- and polysaccharides decreases with pH,[29] there is still a possibility of boronate–sugar interactions in the pH range from 7.5 to 7.9 (see Table 2) and thus the potential for cell adhesion to the BCC-grafted surfaces. The affinity adhesion of the stained yeast cells was studied at different pH values, as illustrated in Figure 9.

The cells adhered quite well to the grafted glasses in the pH range from 8.0 to 9.0, forming a structure almost monolayer in its nature, as shown in Figure 10a. The differences between the optical densities of the adhered layers at different pH values were within the limits of experimental error, and the cell layers were stable during extensive rinsing with 0.5 M sodium bicarbonate or 0.5 M sodium phosphate buffer solutions but could be disintegrated by rinsing with 0.1 M fructose solution in the above buffers. At pH 7.8 the population of the surface by cells was somewhat poorer than in the above pH range (see Figure 10b) and the stability of the adhered layer was lower, with a slow leakage of cells being noticeable during rinsing of the glass support with 0.5 M sodium phosphate buffer (pH 7.8). Similar instability of the adhered layer was observed at pH 7.5, whereas at pH 7.0 only rare single cells could be found in the attached state (see Figure 10c).

The above conditions of attachment and detachment of the stained cells suggest the margins within which the boronate-containing polymer brushes can be regarded as synthetic constructs applicable to cell separation. Concerning the pH values at which living animal cells can be manipulated, one can refer to the slightly alkaline medium of pH 8 acceptable for living vascular smooth muscle cells,[37] rat mammary tumor cells,[38] and fish spermatozoa.[39] Owing to the huge variety of cell types displaying different viabilities in nature, the slightly alkaline media should probably be acceptable for many cell types.

Ionic strength is another parameter important for viability of cells. To avoid the adverse effects of changes in intracellular osmotic pressure, one should keep the ionic strength of the extracellular media at about 0.15 M. The profiles of spontaneous and fructose-assisted detachment of the stained cells obtained in 10 mM sodium phosphate buffer containing 0.15 M NaCl (pH 8.0) are illustrated in Figure 9. The lower ionic strength resulted in easier detachment of the weakly adsorbed cells and sparse population of the surface by the adhered cells relative to that observed in 0.5 M sodium phosphate buffer at the same pH 8.0, probably owing to a diminished salting-out effect of the buffer. Nevertheless, adhesion of the stained cells to the copolymer-grafted support was well expressed, the cell layer was relatively stable, and the fructose-assisted cell detachment was easily achieved.

Regeneration of the DMAA-NAAPBA-grafted glass plates and their operational stability: Detachment of agarose particles and yeast cells from the DMAA-NAAPBA-grafted glass plates was achieved thanks to complex formation between the boronate groups and fructose, a sugar with high affinity to boronates,[32] as described above. Repeated sedi-

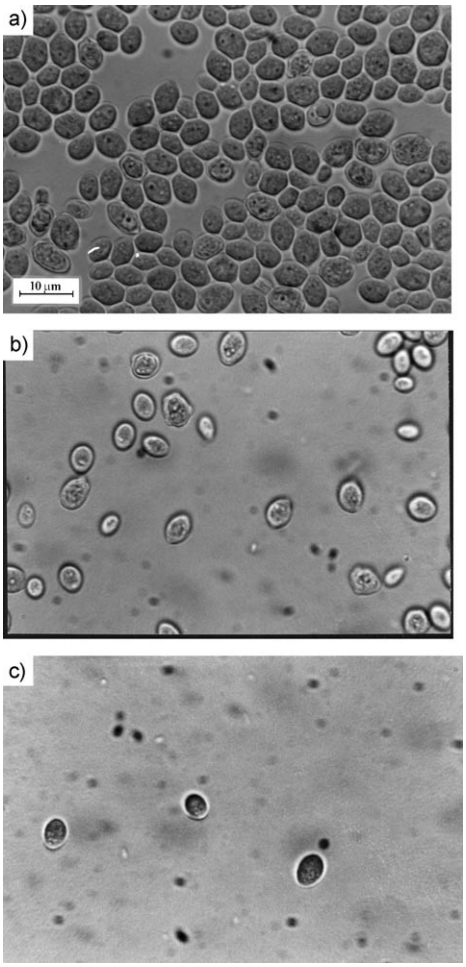


Figure 10. Micrographs of the DMAA-NAAPBA-grafted glass plates (25×45 mm) with adhered stained yeast cells: a) at pH 9.0, b) pH 7.8, c) pH 7.0. Time of contact 1 h at each pH.

mentation either of agarose particles or of the stained yeast cells onto used polymer-grafted plates rinsed with 0.5 M sodium bicarbonate buffer (pH 9.0) resulted in almost zero adhesion: obviously a regeneration procedure for the polymer-grafted support was required. It was found that incubation of the glass plates in 0.05 M acetic acid (pH 3.3) at room temperature for 30 min, followed by overnight incubation in distilled water and equilibration with 0.5 M sodium bicarbonate buffer, allows for almost complete regeneration of the adhesive properties of the polymer-grafted supports. Presumably, their contact with the acidic medium resulted in dissociation of boronate-fructose complexes, and liberation of the sugar from the polymer brush into the contacting solution. Longer contacts of the supports with the acidic medium (up to 24 h) did not improve the cell and particle adhesion, while a shorter contact time (<10 min) resulted in incomplete restoration of the adhesive properties.

Figure 11 illustrates the profiles of spontaneous and fructose-assisted yeast cell detachment obtained in several cycles of cell adhesion, detachment, and regeneration procedures. It is worthwhile to note that the stability of the adhered cell layer remained virtually unchanged after five cycles, although the binding capacity of the support was slowly decreasing. After eight cycles leakage of the adhered cells became noticeable, although the grafted support was still quite capable of adhesion and quantitative release of the cells, controlled by the addition of fructose.

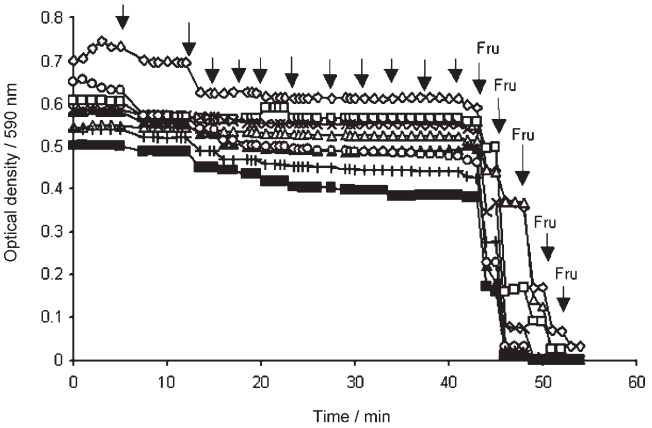


Figure 11. Spontaneous and fructose-assisted detachment of the stained yeast cells from the glass plates grafted with DMAA-NAAPBA copolymer in several sequential cycles of cell adhesion and detachment: 1st cycle (◇), 2nd cycle (□), 3rd cycle (△), 4th cycle (▲), 5th cycle (*), 6th cycle (○), 7th cycle (+), 8th cycle (■), pH 9.0. The cell suspension was allowed to contact the glasses for 1 h in each cycle. Arrows indicate the addition of a portion of fresh buffer and turning upside-down of a closed cuvette containing the glass support. Decimolar fructose in the buffer was used for cell detachment.

Although a competitive affinity elution of adhered yeast cells with dextran as a competitor had been described earlier,[40] no data had been reported on the possibility of carrier regeneration. The cells could not be detached quantitatively by addition of the competitor under the conditions of shear stress allowing for existence of a stable adhered cell layer. Preincubation of the cell layer with dextran-containing buffer, however, enhanced the fraction of detached cells at a given shear stress.[40] In the current study, the addition of fructose at concentrations higher than 0.03 M allowed quantitative detachment of the adhered cell layer, whereas this layer was stable in the absence of the competitor.

The use of monovalent ligands for the competitive elution of bound cells was previously considered to be impractical,[7,41] according to the theoretical model[41] assuming equal binding strengths of the competitor and the immobilized ligand to the cell receptors. In our case, however, the binding constant of fructose to boronate is about two orders of magnitude greater than that of the cell receptor (mannose units of the cell wall mannan oligosaccharides). This might be a reason for the efficient displacement of the receptor from its complexes with boronates, resulting in the detachment of cells. Another reason might be the high diffusivity of fructose, making it capable of penetration into the sterically constrained contact area between the cells and the brush of DEAE-NAAPBA copolymer. We believe that low-molecular-weight competitors exhibiting high association rates[42] and affinities to the immobilized ligands, thus pre-

vailing over those of the cell receptors can assist a quick and mild cell detachment.

Conclusions

We have prepared glass plates and capillary tubes end-grafted with a boronate-containing copolymer of *N*,*N*-dimethylacrylamide. In the soluble state, a copolymer of similar composition spontaneously adsorbed to cross-linked agarose gel at pH 7.5, due to specific boronate–sugar interactions. In turn, the agarose particles readily adhered to the polymer brushes of a boronate copolymer but could be quantitatively detached in the presence of fructose, a sugar that strongly competes with agarose polysaccharide for binding to the boronates. A similar adhesion pattern was displayed by the stained yeast cells, which formed stable, monolayer-like structures on the surfaces of the copolymer-grafted supports. The cell adhesion and stability of the attached cell layer increased with pH and, therefore, with the degree of ionization of the boronate-containing copolymer. The grafted siliceous supports allowed several cell adhesion and detachment cycles. Because of the wide variety of oligo- and polysaccharides localized in the walls of different microbial and animal cells, the described copolymer grafting and particle adhesion techniques might be useful for the development of new cell separation methods. Recently, a preparative manipulation of gold nanoparticles based on boronate–diol interactions has been reported.[43] To the best of our knowledge the current study is the first one to have demonstrated potential for affinity adhesion of micron-sized particles followed by triggering of their quantitative detachment by the introduction of a low-molecular-weight affinity competitor.

Experimental Section

Materials: 1,4-Dioxane, sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium chloride, hydrochloric acid, and acetic acid were products of Merck (Darmstadt, Germany). 3-Aminophenylboronic acid hydrochloride, *N*,*N*-dimethylacrylamide, 3-mercaptopropyltrimethoxysilane, 3-glycidoxypropyltrimethoxysilane, and aluminum oxide were products of Aldrich (Steinheim, Germany). 2,2′-Azobis(2-methylpropionitrile) (AMPN) was purchased from Acros (New Jersey, USA). Acryloyl chloride was purchased from Fluka Chemica (Buchs, Switzerland). *N*-Acryloyl-*m*-aminophenylboronic acid (NAAPBA) and poly(*N*,*N*-dimethylacrylamide) were prepared as described elsewhere.[28] The baker's yeast cells were purchased in a local supermarket. Sepharose CL-6B was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden).

Chemical modification of glass plates with 3-glycidoxypropyltrimethoxy silane (3-GPTMS) and immobilization of 3-aminophenylboronic acid (3-APBA): The glass plates (9×36 mm) were successively treated at room temperature with NaOH (4 M, 2 days), distilled water, and HCl (4 M, 2 days), washed with distilled water and ethanol, dried under vacuum, and placed in a 3-GPTMS solution (5% v/v, 50 mL) in 1,4-dioxane. The chemical modification was carried out in the boiling solution of 3-GPTMS over about 16 h. The glasses were then rinsed with several portions of fresh 1,4-dioxane (50 mL) and dried under vacuum in a desiccator over dry calcium chloride. Immobilization of 3-APBA was performed by the previously described method.[24] Briefly, the 3-GPTMS-modified glass plates were placed in 3-APBA solution (4% w/v) in water adjusted to pH 8.5 with sodium bicarbonate (0.5 M) and were agitated on a rocking table for 24 h at room temperature. The glass plates were rinsed with several portions of distilled water and stored in water. To hydrolyze the epoxy groups of the 3-GPTMS-glasses into diols, the glass plates were treated with diluted hydrochloric acid (pH 2.4) for 24 h at room temperature.[44] After the hydrolysis the plates were rinsed with distilled water and stored in water.

Chemical modification of glass plates with 3-mercaptopropyltrimethoxysilane (3-MPTMS) and graft copolymerization of DMAA and NAAPBA on the modified glasses: Glass plates (9×36 mm or 25×45 mm) or capillary tubes (10 cm, 1.2 mm inner diameter) were successively treated at room temperature with NaOH (4 M, 2 days), distilled water, and HCl (4 M, 2 days), washed with distilled water and ethanol, and dried under vacuum. The washed plates were treated with 3-MPTMS (2% v/v) in 1,4-dioxane at 100 °C for 24 h, rinsed with the solvent, dried under vacuum, and placed in the polymerization mixture containing NAAPBA (432 mg, 2.27 mmol), DMAA (2.07 mL, 20 mmol), and AMPN (54 mg) in ethanol (20 mL). Free-radical polymerization of the monomers was carried out for 5 h at 75 °C with nitrogen bubbling. The glass plates were rinsed with ethanol, 1,4-dioxane, and ethanol, and dried under vacuum. The graft polymerization of DMAA on the 3-MPTMS-modified glasses was carried out as described above, but with no NAAPBA added to the reaction mixture.

Estimation of DMAA-NAAPBA copolymer adsorption capacity on Sepharose CL-6B at different pH values: Solutions of the copolymer (5 mg mL^{-1}) in sodium phosphate (0.1 M) or sodium bicarbonate (0.1 M) buffer with pH from 7.1 to 9.2 were applied in frontal mode, at a flow rate of 0.3 mL min^{-1}, to Sepharose CL-6B columns (1×2.1 cm) equilibrated with the same buffer. After saturation had been reached, the columns were washed with the buffer solution to remove any weakly adsorbed copolymer fraction. Elution of the adsorbed copolymer was performed with fructose (10 mM) in the same buffer. Fractions (1 mL) were collected and monitored at 245 nm (boronate group absorption) or 220 nm (amide group absorption, for poly-*N*,*N*-dimethylacrylamide). Copolymer adsorption capacity (C_1) was calculated from the area between the breakthrough curves of DMAA-NAAPBA copolymer and poly-*N*,*N*-dimethylacrylamide (the latter exhibited no adsorption on Sepharose). Copolymer adsorption capacity (C_2) was calculated from the peak area of the fructose eluate.

Measurements of capillary rise in glass tubes and estimation of water contact angles: The chemically modified and unmodified glass capillary tubes were vertically positioned in a thermostated test-tube so that their lower ends were immersed in distilled water at a given temperature and the upper ends were open to the air. The capillary rise was estimated with a ruler. With assumption of $\cos\theta = 1$ ($\theta = 0$) for water contact with the NaOH- and HCl-treated glass capillaries, the average inner diameter of the capillary glass tubes (10 samples) was calculated as 1.2± 0.1 mm from Equation (1).[45] and surface tension of water γ equal to 71.97 mN m^{-1} at 25 °C.[46]

$$h = 2\gamma\cos\theta/\rho gr \quad (1)$$

The advancing contact angles of water with the inner surfaces of the chemically modified capillaries (5 samples) were calculated from the values of capillary rise at 25 °C according to the above formula. A modified capillary was kept in contact with water for 1 h (the time needed for equilibration of the capillary rise) and the capillary rise was measured at intervals.

The contact angles of water with the modified glass plates were also determined by axis symmetrical drop shape analysis in a sessile drop configuration.[47] A droplet of distilled water (4 μL) was deposited on the modified glass plate mounted on a horizontal thermostated support covered with a windowed chamber containing wet cotton wool to minimize droplet evaporation. The droplet was video recorded and the droplet volume, area, and contact angle were registered once a second for about 10 min. Three to four independent contact angle measurements were performed with different modified glass plates.

Staining of the yeast cells with Procion Red HE-3B—cell counting: Yeast cells were suspended in NaCl (0.15 M, 20 mg mL^{-1}) and the mixture was heated for 40 min at 70 °C. The cells were washed twice with distilled water, separated by centrifugation (3700 × g, 15 min), and resuspended in distilled water (5 mL). The Procion Red dye (30 mg) and NaCl (0.3 g) were added to the suspension of yeast cells and agitated on a rocking table for 24 h. Sodium carbonate (30 mg) was added to the suspension, which was kept on agitating for another 36 h on the rocking table. The cells were washed several times with distilled water and separated by centrifugation (3700 g, 5 min) until the washings became colorless. The stained cells were resuspended in sodium bicarbonate buffer (0.5 M, pH 9.05) and stored in the buffer, at a concentration of about 100 mg mL^{-1}. The number of stained cells per 1 mL of the prepared suspension was estimated as 2×10^8 by counting in a hemocytometer, after 100-fold dilution with the sodium bicarbonate buffer.

Sedimentation of the stained yeast cells onto the modified and unmodified glass plates and estimation of optical density: A glass plate was rinsed with sodium bicarbonate buffer (0.5 M, pH 8.5–9.0) or with sodium phosphate buffer (0.5 M, pH 7.0 to pH 8.0) and placed in the suspension (10 % w/v) of stained yeast cells in the same buffer. The cells were allowed to sediment on a horizontally mounted glass for a given time from 0.5 min to 2 h, the depth of the liquid above the surface being about 1 cm. The sedimentation velocity of the cells under these conditions was about 2 mm h^{-1}. The glass was rinsed with a stream of the buffer solution (3 mL) from a glass pipette, transferred to a spectrophotometic cuvette (4 mL) containing the same buffer (3 mL) and an air bubble (ca. 0.5 mL), and turned upside down twice in order to remove the unattached or poorly attached cells. The optical density of the cells attached to the glass was quantified with a spectrophotometer (λ_{max} = 590 nm), the glass plate being placed vertically in a 1 cm cuvette. After a few minutes, the cuvette containing the plate was again turned upside down twice and the buffer solution was decanted. Fresh buffer was added into the cuvette and the optical density of attached cells was quantified again. To detach the adhered cells from the glasses, rinsing with the buffer containing fructose (5 to 100 mM) was carried out and the optical density was measured in the above manner.

Regeneration of DMAA-NAAPBA-grafted glasses: To regenerate DMAA-NAAPBA-grafted glasses, they were placed in acetic acid (0.05 M, pH 3.3) at room temperature for 0.5 h, rinsed with distilled water, and stored in distilled water before the next cycle of cell attachment.

Acknowledgements

This research was supported by the Swedish Competence Center for Bioseparation (CBioSep), the Swedish Research Council (project VR621–2005–3604), the Swedish Foundation for Strategic Research (Area of Chemistry and Life Sciences) and INTAS (projects 0673 and 4813).

[1] W. Lee, K. Saito, S. Furusaki, T. Sugo, *Biotechnol. Bioeng.* **1997**, *53*, 523–528.
[2] W. Lee, S. Furusaki, K. Saito, T. Sugo, K. Makuuchi, *Biotechnol. Prog.* **1996**, *12*, 178–183.
[3] A. Terada, A. Yuasa, S. Tsuneda, A. Hirata, A. Katakai, M. Tamada, *Colloids Surf. B* **2005**, *43*, 99–107.
[4] H. J. Kaper, H. J. Busscher, W. Norde, *J. Biomater. Sci. Polym. Ed.* **2003**, *14*, 313–324.
[5] L. K. Ista, V. H. Perez-Luna, G. P. Lopez, *Appl. Environ. Microbiol.* **1999**, *65*, 1603–1609.
[6] S. K. Hendricks, C. Kwok, M. Shen, T. A. Horbett, B. D. Ratner, J. D. Bryers, *J. Biomed. Mater. Res.* **2000**, *50*, 160–170.
[7] X. Cao, R. Eisenthal, J. Hubble, *Enzyme Microb. Technol.* **2002**, *31*, 153–160.
[8] A. Kumar, A. Rodrigues-Caballero, F. M. Plieva, I. Yu. Galaev, K. S. Nandakumar, M. Kamihira, R. Holmdahl, A. Orfao, B. Mattiasson, *J. Mol. Recognit.* **2004**, *17*, 1–11.
[9] E. B. Zhulina, O. V. Borisov, V. A. Pryamitsyn, T. M. Birshtein, *Macromolecules* **1991**, *24*, 140–149.
[10] T. Okano, N. Yamada, M. Okuhara, H. Sakai, Y. Sakurai, *Biomaterials* **1995**, *16*, 297–303.
[11] C. de las Heras Alarcon, T. Farhan, V. L. Osborne, W. T. S. Huck, C. Alexander, *J. Mater. Chem.* **2005**, *15*, 2089–2094.
[12] D. E. Brooks, C. A. Haynes, D. Hritcu, B. M. Steels, W. Müller, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7064–7067.
[13] Y. Hirano, M. Okuno, T. Hayashi, K. Goto, A. Nakajima, *J. Biomater. Sci. Polym. Ed.* **1993**, *4*, 235–243.
[14] D. Cox, J. Seki, *Thromb. Res.* **1998**, *91*, 129–136.
[15] D. Zopf, S. Ohlson, *Nature* **1990**, *346*, 87–88.
[16] S. Ohlson, M. Bergström, L. Leickt, D. Zopf, *Bioseparation* **1998**, *7*, 101–105.
[17] V. M. Martin, C. Siewert, A. Scharl, T. Harms, R. Heinze, S. Ohl, A. Radbruch, S. Miltenyi, J. Schmitz, *Exp. Hematol.* **1998**, *26*, 252–264.
[18] M. T. Madigan, J. M. Martinko, J. Parker, *Brock Biology of Microorganisms*, 9th ed., Prentice Hall, Upper Saddle River, **2000**, pp. 69–74.
[19] *Essentials of Glycobiology* (Eds.: A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart, J. Marth), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, **1999**, pp. 285–304.
[20] A. Bergold, W. H. Scouten in *Solid Phase Biochemistry. Analytical and Synthetic Methods* (Ed.: W. H. Scouten), Wiley, London, **1983**, pp. 149–187.
[21] N. D. Winblade, I. D. Nikolic, A. S. Hoffman, J. A. Hubbell, *Biomacromolecules* **2000**, *1*, 523–533.
[22] E. Uchimura, H. Otsuka, T. Okano, Y. Sakurai, K. Kataoka, *Biotechnol. Bioeng.* **2001**, *72*, 307–314.
[23] A. E. Ivanov, D. Szul, R. Hallmann, I. Yu. Galaev, B. Mattiasson, Boronate-containing copolymers as carbohydrate-specific reagents and regulators of cell adhesion, FBPS 05, *6th International Symposium on Frontiers in Biomedical Polymers*, Granada, Spain, June 16–19, **2005**, Book of Abstracts, P-9.
[24] M. Glad, S. Ohlson, L. Hansson, M. O. Mansson, K. Mosbach, *J. Chromatogr.* **1980**, *200*, 254–260.
[25] D. L. Huber, R. P. Manginell, M. A. Samara, B.-I. Kim, B. C. Bunker, *Science* **2003**, *301*, 352–354.
[26] A. E. Ivanov, J. Ekeroth, L. Nilsson, B. Mattiasson, B. Bergenståhl, I. Yu. Galaev, *J. Colloid Interface Sci.* **2006**, *296*, 538–544.
[27] J.-L. Zhang, W. Li, Y. Zhai, H. Yang, Y.-P. Wang, *Appl. Surf. Sci.* **2005**, *245*, 94–101.
[28] A. E. Ivanov, H. Larsson, I. Yu. Galaev, B. Mattiasson, *Polymer* **2004**, *45*, 2495–2505.
[29] K. Shiomori, A. E. Ivanov, I. Yu. Galaev, Y. Kawano, B. Mattiasson, *Macromol. Chem. Phys.* **2004**, *205*, 27–34.
[30] B. Mahltig, H. Böttcher, *J. Sol-Gel Sci. Technol.* **2003**, *27*, 43–52.
[31] D. J. Graves, Y.-T. Wu, *Methods Enzymol.* **1974**, *34*, 140–163.
[32] R. van den Berg, J. A. Peters, H. van Bekkum, *Carbohydr. Res.* **1994**, *253*, 1–12.
[33] G. G. S. Dulton, *Polysaccharides, in Encyclopedia of Polymer Science and Engineering*, Wiley, N.Y., Chichester, **1988**, V. 13, pp. 121–123.
[34] M. P. Nicholls, P. K. C. Paul, *Org. Biomol. Chem.* **2004**, *2*, 1434–1441.
[35] *Gel Filtration. Principles and Methods*, Pharmacia LKB Biotechnology, Uppsala, Sweden, 5th ed., **1991**, p. 41.
[36] M. G.Voronkov, V. P. Mileshkevich, Yu. A. Yuzhelevskii, *The Siloxane Bond. Physical Properties and Chemical Transformations,* Consultants Bureau, N.Y. and London, **1978**.
[37] S. Kalyankrishna, J.-H. Parnmentier, K. U. Malik, *Prostaglandins & Other Lipid Mediators* **2002**, *70*, 13–29.
[38] H. von Euler, A. Söderstedt, A. Thörne, J. M. Olsson, G. Yongqing, *Bioelectrochemistry* **2002**, *58*, 163–170.
[39] M. Segovia, J. A. Jenkins, C. Paniagua-Chavez, T. R. Tiersch, *Theriogenology* **2000**, *53*, 1489–1499.
[40] A. Lam, X. Cao, R. Eisenthal, J. Hubble, *Enzyme Microb. Technol.* **2001**, *29*, 28–33.
[41] J. Hubble, *Immunol. Today* **1997**, *18*, 305–306.

[42] C. E. Orsello, D. A. Lauffenburger, D. A. Hammer, *Trends Biotechnol.* **2001**, *19*, 310–316.
[43] O. Abed, A. Vaskevich, R. Arad-Yellin, A. Shanzer, I. Rubinstein, *Chem. Eur. J.* **2005**, *11*, 2836–2841.
[44] F. E. Regnier, R. Noel, *J. Chromatogr. Sci.* **1976**, *14*, 316–320.
[45] A. W. Adamson, *Physical Chemistry of Surfaces*, Wiley, Chichester, **1990**, p. 12.
[46] *CRC Handbook of Chemistry and Physics* (Eds.: D. R. Lide, H. P. R. Fredirkse), CRC Press, Boca Raton, London, **1995**, pp. 6–10.
[47] P. Chen, D. Y. Kwok, R. M. Prokop, O. I. del Rio, S. S. Susnar, A. W. Neumann, in *Drops and Bubbles in Interfacial Research* (Eds.: D. Möbius, R. Miller), Elsevier, Amsterdam, **1998**, pp. 61–90.

Received: March 10, 2006
Published online: July 18, 2006

