Temperature-Controlled Adhesion to Carbohydrate Functionalized Microgel Films: An E. coli and Lectin Binding Study

Tanja J. Paul, Alexander K. Strzelczyk, and Stephan Schmidt*

The preparation of thermoresponsive mannose functionalized monolayers of poly(N-isopropylacrylamide) microgels and the analysis of the specific binding of concanavalin A (ConA) and E. coli above and below the lower critical solution temperature (LCST) are shown. Via inhibition and direct binding assays it is found that ConA binding is time-dependent, where at short incubation times binding is stronger above the LCST. Given larger incubation times, the interaction of ConA to the microgel network is increased below the LCST when compared to temperatures above the LCST, possibly due to increased ConA diffusion and multivalent binding in the more open microgel network below the LCST. For E. coli, which presents only monovalent lectins and is too large to diffuse into the network, binding is always enhanced above the LCST. This is due to the larger mannose density of the microgel layer above the LCST increasing the interaction to E. coli. Once bound to the microgel layer above the LCST, E. coli cannot be released by cooling down below the LCST. Overall, this suggests that the carbohydrate presenting microgel layers enable specific binding where the temperature-induced transition between swollen and collapsed microgels may increase or decrease binding depending on the receptor size.

1. Introduction

Carbohydrates as a major component of cell surfaces mediate countless cellular processes such as adhesion, cell–cell communication, signal transduction, or fertilization.\(^1,^2\) Also pathogenic bacteria can harness carbohydrates at the cells glycoalx to attach to cells, to colonize, and to start infections.\(^3,^4\) These interactions are mediated by lectins, i.e., receptors at the tip of long hair-like protein complex called fimbriae.\(^5,^6\) A prominent example of pathogens adhering to glycosylated cell surfaces is Escherichia coli (E. coli) via the mannose specific binding site FimH at the tip of the fimbriae. Escherichia coli can cause numerous infectious diseases including neonatal meningitis leading to high fatality rates of new-born children in developing countries.\(^7,^8\) Due to the ongoing antimicrobial resistance crisis making it increasingly harder to fight such infections,\(^10\) the inhibition of bacterial lectins has been proposed as a new treatment strategy.\(^11\) Such inhibitors are based on multivalent natural carbohydrates or glycoconjugates that bind to lectins with high avidity, thereby blocking them resulting in reduced pathogen adhesion and invasion.\(^12-19\)

On the other hand, surface coatings of such glycoconjugates are auspicious for capturing lectins or specific bacteria, e.g. in water purification, for the detection of biological contaminants or for quantifying carbohydrate–cell interactions.\(^20-24\) For practical considerations and to improve the viability of such surfaces, it is desirable to be able to remotely control the binding of bacteria to such surfaces. For example, to capture the bacteria in a first stage to remove the contaminant, and to release the bacteria in a second stage for diagnostics or simply reusing the coating. Several groups reported on the synthesis of remotely switchable thermoresponsive carbohydrate presenting polymer brushes with a lower critical solution temperature (LCST) and tested their binding to bacteria and lectins.\(^25-30\) Upon temperature increase, these polymer brushes collapse thereby the density of the carbohydrate residues is increased while decreasing the polymers' steric repulsion by reducing their excluded volume, which overall increases carbohydrate-receptor binding.\(^31\) Although the capture of lectins and bacteria could be readily switched “on” by raising the temperature above the phase transition temperature of the polymer, the release of bacteria or protein by lowering the temperature was not yet reported perhaps due to the strong hysteresis of the switchable glycopolymer brushes in the bound and collapsed state.\(^12\)

In comparison to polymer brushes, microgels allow quite simple coating procedures, elaborate surface functionalization processes are not needed.\(^33\) Furthermore, when compared to thin polymer brushes, microgel monolayers in water exhibit
thicknesses of several hundred nanometers, and thus mimic the mechanical properties of the cell environment.\cite{34,35} In addition, several groups presented the synthesis of carbohydrate functionalized microgels for the switchable binding of carbohydrate binding proteins.\cite{36-38} Typically, such thermosensitive microgels are composed of poly(N-isopropylacrylamide) or poly(oligo ethylene glycol acrylates).\cite{39} Quite similarly to thermosensitive polymers, by increasing the temperature above the LCST carbohydrate functionalized microgels form polymer–polymer contacts and collapse, thereby decreasing the steric repulsion while increasing the carbohydrate density and the elastic modulus.\cite{34,35,40} which increases E. coli clustering in solution and carbohydrate binding overall.\cite{38,41,42,43} The temperature dependent shifts in the microgels’ elastic modulus and surface roughness can be used to create switchable cell culture surfaces that enable the controlled attachment and detachment of cells even without addressing specific cell adhesion receptors.\cite{35,40,44} However, microgel coatings specifically targeting selected cells, e.g. bacteria via lectin binding have not been studied so far. Therefore, in this work we aim at coatings of mannose functionalized poly(N-isopropylacrylamide) microgels to test their specific temperature dependent binding to E. coli in comparison to a mannose specific model lectin (concanavalin A, ConA). We aim at understanding the underlying mechanisms of lectin and bacterial adhesion at the soft, fuzzy microgel networks as a function of the temperature, microgel swelling degree, and mannose ligand density. The carbohydrate interactions of these coatings are studied via a direct binding assay and an inhibition assay at different incubation times. Finally, the ability to again release bound bacteria from the microgel coatings is tested.

2. Results and Discussion

2.1. Synthesis of Carbohydrate-Functionalized Microgels

By copolymerizing different amounts of the mannose comonomers N-2-(α-D-mannopyranosyloxy)ethylacrylamide, (ManEAM) or N-2-[α-D-mannopyranosyloxy]ethyl)methacrylamide (ManEMAM) with NIPAM and a bifunctional cross-linker, we obtained microgels with varying mannose density in a single step.\cite{38} The mannose functionalization degree in the microgel, i.e., the amount of mannose monomer as compared to NIPAM repeat units, was 0.4 to 0.8 mol%. The samples are termed by the mannose functionalization degree, e.g. Man0.5 signifies microgels with a functionalization degree of 0.5% ManEAM. Man0.4 and Man0.8 describe microgels with a functionalization degree of 0.4% and, respectively, 0.8% ManEMAM. To characterize the hydrodynamic radii and swelling ratio between 20 and 40 °C, dynamic light scattering (DLS) was used (see Table 1). Both microgels with ManEMAM as comonomer have nearly the same hydrodynamic radii and the same swelling ratio although the amount of carbohydrate within the microgels is doubled. It appears that ManEMAM was incorporated into the microgel network at a higher rate as compared to ManEAM, since for Man0.5 and ManM0.8 the same molar ratios of comonomer were used but a significantly larger amount of comonomer was incorporated for ManM0.8. From the polymerization kinetics the opposite behavior could be expected, i.e., acrylamides (ManEAM) polymerize and integrate into the microgel network faster than methacrylamides (ManEMAM). However, the polymerization was conducted above the LCST of PNIPAM where small surfactant-stabilized hydrophobic microgel precursors are formed first.\cite{39} It is likely that the more hydrophobic ManEMAM was incorporated better to these growing microgel precursors as compared to the more hydrophilic monomer ManEAM. Such differences in microgel growth may explain the varying microgel compositions and differences in hydrodynamic radius and swelling ratio.\cite{45} Here, Man0.5 with ManEAM as comonomer was smaller but exhibited a higher swelling ratio as compared to the ManM0.4 and ManM0.8 microgels.

After synthesizing the microgels, the functionalization of microwell plate surfaces was tested to find the optimum microgel density. For consistent results in ConA and E. coli binding assays it is important that the microgel films in the microwells were reproducible. Therefore, micropette surfaces were coated by straightforward drop-casting, which included drying a microgel suspension in the multiwells followed by annealing and washing with the measurement buffer. To find out the optimal microgel surface density, different microgel concentrations (120 µL, 1 to 0.002 mg mL⁻¹) of Man0.5 were drop-cast in a microwell plate and the fluorescence intensity was read out after incubation/washing with E. coli or FITC labeled ConA (see Figure 1). The results showed a plateau of bound ConA and E. coli at around 0.1 mg mL⁻¹ of microgels. Atomic force microscopy (AFM) images showed that at this microgel concentration dense monolayers are formed. The simple drop casting method has been used before to form similar ordered microgel films.\cite{33,44,46} Drop casting of microgel dispersions at concentrations larger than 0.1 mg mL⁻¹ likely leads to multilayers that are removed via incubation and reswelling in water leaving a dense microgel monolayer adhering to the hydrophobic polystyrene surface. Therefore, all following binding assays were conducted with microgel coatings prepared at this microgel concentration and the same drop-casting procedure to assure reproducible microgel monolayers.

| Sample name |  \( R_h \) at 20 °C [nm] (\( R_{300} \)) | Swelling ratio  \( R_{300}/R_{40} \) | Mannose functionalization degree [\( \mu \text{mol g}^{-1} \)] | Mannose functionalization degree [mol% monomer] |
|-------------|--------------------------------|----------------|---------------------------|--------------------------------|
| Man0.5      | 268 ± 28                        | 3.2            | 43 ± 4                    | 0.5                            |
| ManM0.4     | 365 ± 16                        | 2.5            | 32 ± 3                    | 0.4                            |
| ManM0.8     | 361 ± 27                        | 2.3            | 67 ± 5                    | 0.8                            |
2.2. ConA Binding to Microgel Surfaces

Having identified the conditions for microgel coating, microwell plates were prepared for adhesion studies using ConA as a receptor. ConA is a well-known homotetrameric model lectin that binds α-d-mannopyranoside.[47] In the inhibition assay, different amounts of the inhibitor α-d-methyl manno-pyranoside (MeMan) were added to determine the inhibitory concentration (IC50 value, Figure 2, left). IC50 values represent the amount of inhibitor required to reduce the amount of bound receptor by 50% compared to the noninhibited surfaces. This means that at higher IC50 values a larger amount of inhibitor was necessary to inhibit the binding between ConA and the microgel surface, thus the interaction was stronger. Comparing the IC50 values at different temperatures show how the microgel phase transition affects the binding between ConA and the microgel surface. For the direct binding assay the amount of microgel bound FITC-labeled ConA was directly determined by fluorescence microscopy. To directly measure the amount of bound ConA, microscope-based fluorescence measurements were carried out on microgel layers adsorbed to the same polystyrene microwell plates used for the inhibition assay (Figure 2, right). The inhibition assay shows how strong ConA was bound to the microgel surfaces whereas the direct binding assay shows how much ConA was bound to the microgel surfaces.[48]

2.2.1. Inhibition Assay

The thermoresponsive specific binding between mannose bearing microgel surfaces and ConA was analyzed with the inhibition assay by incubation with FITC-ConA and a MeMan solution series at 20 and 40 °C for either 30 min or 24 h. The surfaces were washed and the amount of bound FITC-ConA was determined by reading the fluorescence signal with a plate reader. The resulting inhibition curves yield the IC50 values and the Hill coefficient, the slope of the curve, which is a measure for the degree of binding cooperativity (Figure 3).[49,50] In this experimental setup higher IC50 values would indicate that more MeMan was required to inhibit the ConA-microgel adhesion. This could be the case for a higher mannose ligand density on the surface.[51,52]

For a ConA incubation time of 30 min the IC50 values were larger above the LCST (Figure 3) indicating stronger binding due to a larger mannose density in the collapsed state. However, at incubation times of 24 h the IC50 values show the opposite behavior, where stronger ConA-microgel binding was observed below the LCST. Furthermore, at 24 h incubation the slopes of the inhibition curves as signified by the Hill coefficients were clearly smaller below the LCST, which suggests that the degree of cooperative ConA binding to the microgel layer was larger below the LCST. A clear mannose functionalization degree dependence was observed only at small incubation times.
where increased mannose functionalization degrees resulted in larger IC\textsubscript{50} values and stronger binding. Such concentration dependence on ligand units is expected due to statistical effects, i.e., rapid binding and rebinding of ConA due to the short lifetime of single carbohydrate–lectin complexes.[18,53–55] The fact that this mannose concentration dependence was not seen at long incubation times hints at multiple simultaneous (chelate-like) mannose interactions at single ConA molecules where statistical effects do not dominate. Therefore, the inhibition assay suggests that binding of ConA to the microgel layer requires long incubation times to reach an equilibrium. Initially, ConA binding to collapsed microgels above the LCST was stronger compared to swollen microgels below the LCST. Given longer incubation times below the LCST, it seems that ConA was able to bind additional mannose units via multivalent binding in the more flexible microgel network. This is different above the LCST, where the microgels are collapsed and rigid, thus the degree of multivalent cooperative binding was lower. The large IC\textsubscript{50} value for ManM0.4 below the LCST for long incubation suggests stronger binding to the microgel network compared to ManM0.8 and Man0.5 with a higher functionalization degree. This could be explained by an increased diffusion of ConA into the ManM0.4 network, perhaps due to slowed binding to the network since the density of Man units was smaller compared to the other microgels. At a higher mannose density for ManM0.8 and Man0.5, the diffusion of ConA could be reduced due to stronger binding of the protein near the microgel surface, thus blocking further diffusion into the network.[56] Therefore more inhibitor is required to remove ConA in ManM0.4 where ConA might be bound deeper within the microgel network.

2.2.2. Direct Binding Assay

To directly compare the amount of bound ConA to the microgels surfaces, the fluorescence intensity of ConA-FITC of the microgel surface was determined by optical microscopy above and below the LCST at different incubation times (Figure 4). Microscope based fluorescence analysis showed that more ConA was bound to microgels at larger functionalization degrees. Significantly more ConA binds after 24 h incubation, which again hints at long equilibration times. Notably, raising the temperature above the LCST resulted in an increase in bound ConA. However, when compared to the large changes in IC\textsubscript{50} upon temperature increase, (Figure 3) these effects were small.

We interpret these findings by delayed diffusion of the ConA molecules into the “fuzzy,” less cross-linked corona of the microgel particles. Such radial cross-linking density gradients are due to the larger polymerization rate of the bifunctional cross-linker when compared to NIPAM and the mannose comonomers. The mesh width of the swollen microgels is on the order of 10 nm[57,58] allowing ConA to diffuse into the microgel network below the LCST. For short incubation times where diffusion is incomplete, the ConA binding to microgels below the LCST was small and the binding can be readily inhibited by the addition of MeMan since the ConA molecules could only bind the surface-exposed mannose ligands (Figure 5, left). For collapsed microgels it is likely that the accessibility of mannose units is increased due to the reduced steric repulsion of the network and the potential enrichment of the hydrophilic mannose units at the surface of the hydrophobic layer. Thus, for short incubation times the amount of bound ConA and

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Inhibitory concentration (IC\textsubscript{50}) values and Hill coefficients measured for microgel coated surfaces using Man0.5, ManM0.4, and ManM0.8 after 30 min incubation (left) and after 24 h incubation (right) using ConA as a receptor. Blue bars represent measurements at 20 °C and red bars at 40 °C.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Fluorescence intensities measured of FITC-ConA bound to microgel surface at 20 °C (blue bars) and 40 °C (red bars) after 30 min (left) and 24 h (right) incubation.
IC$_{50}$ values were larger above the LCST. When increasing the incubation time, the ConA molecules were allowed to diffuse into the microgel corona below the LCST enabling multivalent binding due to the flexible network as signified by increased IC$_{50}$ values (Figure 5, right). However, in the collapsed state above the LCST such diffusion into the gel is not possible and the network is more rigid reducing multivalent binding and lower IC$_{50}$ values.

### 2.3. *Escherichia coli* Binding to Microgel Surfaces

To show the ability to capture carbohydrate binding bacteria by temperature stimulus on microgel coatings, we investigated the adhesion of *E. coli* as a well-known mannose binding bacterium. The *E. coli* adhesion was again quantified by fluorescence-based microplate reading using the green fluorescence protein tagged type 1-fimbriated strain pKL1162.\cite{59} The inhibition assays were conducted like the ConA binding assays. For the direct binding assay the amount of adhered bacteria was determined using a plate reader instead of an optical microscope to increase the size of the sampled area.

#### 2.3.1. Inhibition Assay

For an incubation time of 30 min, the comparison of IC$_{50}$ values at 20 and 40 °C showed only small effects when crossing the LCST and the Hill coefficients were close to unity as expected for the monovalent FimH receptor (Figure 6). It is likely that an incubation time of 30 min was too small to observe clear effects given the small diffusion constants of the large *E. coli* bacteria compared to the molecular-sized ConA.

After an incubation time of 24 h the changes in IC$_{50}$ values were similar for all microgel surfaces and a clear temperature response was observed. All microgel surfaces showed an increase in IC$_{50}$ values at elevated temperature, i.e., more inhibitor was required to remove the bacteria from the surfaces at 40 °C. This was caused by an increase in mannose density above the LCST compared to the microgel film below the LCST. An increase in the surface density of binding motifs often correlates with increasing IC$_{50}$ values.\cite{51} In case of weak carbohydrate binding, higher rates of rebinding events at higher densities of ligands may explain this effect.\cite{52,60} For collapsed microgels above the LCST, the accessibility of mannose units might be improved at the surface which would also lead to increased binding.\cite{31}

#### 2.3.2. Direct Binding Assay

A microscope-based read out for *E. coli* adhesion toward the microgel surface was unsuitable due to the small areas covered. Therefore, the fluorescence signals were collected using a microplate reader. The data showed that for short incubation times of 30 min the adhesion of *E. coli* varied significantly, perhaps due to the slow diffusion of the bacteria compared to ConA. Therefore, here only the results for long adhesion times (24 h) are shown (Figure 7). The results show that for microgels with low mannose density (ManM0.4 and Man0.5) more *E. coli* were bound above the LCST. This might again indicate an increase in the mannose density and improved accessibility of mannose above the LCST due to the collapse of the microgels. For ManM0.8 there was no difference in the number of adhered bacteria when changing the temperature. This suggests that the density of mannose units

---

**Figure 5.** Schematic presentation of the surface of the microgel layer where binding and diffusion of ConA molecules is controlled by a loosely cross-linked network (microgel corona). For short diffusion times binding is overall lower below the lower critical solution temperature (LCST) due to steric repulsion and low mannose accessibility (left). For long incubation times ConA can diffuse in the swollen network and bind multiple mannose units due to the flexibility of the network. Such multivalent binding is reduced in the collapsed state due to the more rigid network.

**Figure 6.** IC$_{50}$ values and Hill coefficients measured for *E. coli* binding to Man0.5, ManM0.4, and ManM0.8 coated surfaces after 30 min incubation (left) and after 24 h incubation (right). Blue bars represent measurements at 20 °C and red bars at 40 °C.
was sufficiently large below the LCST to bind a large number of bacteria.

### 2.4. Releasing E. coli from the Microgel Coatings

The temperature-dependent binding of E. coli to the Man0.5 microgel surfaces was shown to be stronger in the collapsed state above the LCST when compared to the swollen state of the microgel below the LCST (Figure 7). We also studied whether the bound bacteria in the collapsed state of the microgels can be released by washing with buffer solutions below the LCST (Figure 8). This is especially interesting for the surfaces that were incubated with E. coli at 40 °C for 24 h as it was seen that in this case the collapse of the microgel leads to an increased adhesion of the bacteria. We found that washing with cold buffer (below the LCST) showed no significant decrease of the fluorescence intensity in comparison to the surfaces that were washed with warm buffer (above the LCST). Therefore, the reswelling of the microgels upon cooling was not sufficient to release bound bacteria by a substantial degree owing to a strong swelling hysteresis, persistent specific mannose FimH binding, or entanglements between the microgel network and the bacterial fimbria.

![Figure 7](image-url) Direct E. coli binding adhesion assay. a) Fluorescence intensities measured on microgel surfaces after 24 h incubation. Blue bars represent measurements at 20 °C and red bars at 40 °C. b) Typical microgel surfaces after E. coli incubation for 24 h.

![Figure 8](image-url) Relative fluorescence intensity after washing the E. coli incubated surfaces (24 h, 40 °C) with cold (20 °C, left) and warm buffer (40 °C, right).

### 3. Conclusion

PNIPAM microgel monolayer films decorated with mannose units were prepared on polystyrene microwell surfaces to study the binding of ConA and E. coli below and above the LCST of PNIPAM for short (30 min) and long incubation times (24 h). For long incubation times it was shown that when increasing the temperature above the LCST the interaction of E. coli with the microgel layer as measured by IC50 values was increased. Also the number of bound E. coli was increased. This is explained by a higher mannose density and reduced steric repulsion in the collapsed state of the microgel layer above the LCST. In contrast to strong E. coli binding above the LCST, for ConA the interaction to the microgel layer after 24 h incubation was stronger below the LCST. This might be explained by multivalent binding of the tetrameric receptor to the flexible swollen polymer network, which is not possible for E. coli since its receptor (FimH) is monovalent and the bacteria is too large to diffuse into the microgel network. For short incubation time the ConA binding was weaker below the LCST perhaps due to delayed diffusion of ConA in the soft swollen microgel corona. Reswelling of the microgels upon cooling was not sufficient to release bound receptors by a substantial degree owing to a strong swelling hysteresis or persistent specific binding. Overall, these results show that the capture of E. coli via ligand decorated microgel layers can be significantly increased above the LCST whereas this was not possible for small multivalent receptors. Furthermore, the temperature dependent swelling of the microgel layer could be combined with other stimuli responsive residues, e.g. light or chemical stimuli to enable coatings with more controlled capture and release capabilities.

### 4. Experimental Section

**Materials:**  N-isopropylacrylamide (NIPAM) (99%), N,N′-methylenebisacrylamide (MBA) (99%), ethanol (≥99.8%), acetic acid (99.8-100%), t-toluic acid (98%), phosphate buffered saline tablets (PBS), sodium methoxide (≥97.5%), Amberlite IR 120 (hydrogen form), acetic anhydride (99.5%), manganese chloride (99.9%), sulfuric acid (95–98%), ammonium persulfate (APS) (98%), LB Broth (Miller, powder microbial growth medium), ethanolamine (≥98%), dichloromethane
Microgel Coating: For coating of the 96-well plates 120 µL of each solution, 125 µL of a 0.5 wt% microgel solution in ultrapure water were prepared. Afterward 625 µL of microgel in ultrapure water were added to the reaction solution to start the polymerization. During the reaction the solution was continuously purged with nitrogen and stirred at 350 rpm. The reaction was stopped after 35 min by cooling with an ice bath. After filtration by glass wool, the reactants were removed by repeated centrifugation at 10 000 g and washing with water.

Phenol-Sulfuric Acid Method: The phenol sulphuric acid method was used to quantify the number of glycomonomers within the microgel network. First, a calibration curve was established using previously reported procedures. Using a stock solution of 320 × 10−6 M phenol and 300 × 10−6 M sulfuric acid, the absorbance of each glycomonomer was determined at 550 nm and plotted against the concentration. The absorbance of the unknown samples was then measured and compared to the calibration curve. The concentration of the glycomonomers was calculated using a standard curve obtained from the calibration.

Dynamic Light Scattering (DLS): Measurements were performed on a Malvern NanoZS (Malvern Panalytical, Kassel, Germany) using a He-Ne laser as the light source. The samples were prepared by diluting the polymer solutions to concentrations of 0.1, 0.5, and 1.0 mg mL−1. The scattered light was detected using a 90° detector and the data were analyzed using the Malvern software. The average hydrodynamic radius (Rh) and polydispersity index (PDI) were calculated from the autocorrelation functions.

Supporting Information: Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements: The authors thank Maria Breuer for performing the NMR measurements and Stephanie Scheelen for performing the SEC measurements. The authors acknowledge funding by the German Research foundation (DFG) in the project SCHM 2748/5-1.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Research data are not shared.

Keywords: biomimetic hydrogels, glycocalyx, lower critical solution temperature, microgel coatings, multivalency, PNIPAM, responsive material

Received: November 13, 2020
Revised: December 16, 2020
Published online: February 19, 2021
