On the influence of the architecture of poly(ethylene glycol)-based thermoresponsive polymers on cell adhesion

Katja Uhlig,^{1,a)} Björn Boysen,^{1,b)} Andreas Lankenau,^{1,c)} Magnus Jaeger,¹
Erik Wischerhoff,² Jean-François Lutz,^{2,d)} André Laschewsky,²
and Claus Duschl¹

¹Fraunhofer Institute for Biomedical Engineering (IBMT), Potsdam, Germany

²Fraunhofer Institute for Applied Polymer Research (IAP), Potsdam, Germany

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Thermoresponsive polymer surface coatings are a promising tool for cell culture applications. They allow for a mild way of cell detachment that preserves the activity of membrane proteins—a prerequisite for reliable cell analysis. To enlarge the application range of these coatings to cells with different adhesion properties, we synthesized various novel poly(ethylene glycol)-based thermoresponsive polymers and describe how (i) their chemical structure and (ii) their surface density affect their efficiency. In order to quantify the influence of both factors, the time for cell spreading and rounding efficiency were observed. As a result, efficiency of cell rounding, which is closely correlated to cell detachment, is less affected by both factors than the time needed for cell spreading. This time can effectively be adjusted by the molecular architecture which includes the length of the polymer backbone and the side chains. Based on this work, recommendations are given for future optimization of functionality of thermoresponsive polymer coatings for cell culture applications. © 2012 American Institute of Physics.

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I. INTRODUCTION

A complex pattern of numerous chemical and physical cues provides the signals required for the control of cellular behavior and cell fate in an organism. In order to make use of cells for applications in areas like regenerative medicine, stem-cell research, medical implants, and biotechnology, a similarly high degree of control over their behavior is prerequisite for successful approaches. Adherent cells respond very sensitively to surface properties, and their behavior can be manipulated by specific surface architectures. Functional elements or properties of such architectures include molecular groups,¹ topography,² or mechanical stiffness.³ A technically highly relevant issue is the control of cell adhesion on substrates for cultivation. Ever since 1950s, cell detachment in animal cell culture has routinely been brought about by enzymatic treatment, e.g., trypsin. However, the disadvantage of this, still widely used approach, is that it non-specifically cleaves membrane proteins.⁴ As a consequence, this method severely limits the sensitivity of various cell analyzing techniques, such as patch clamp or membrane-based flow cytometry. In recent years, it became clear that the use of thermoresponsive polymer (TRP) coatings is a valuable non-invasive alternative that can substitute the harsh enzyme treatment. At the lower critical solution temperature (LCST), the conformation of these polymers changes reversibly,⁵ thus altering the surface properties and, as a result, cell adhesion in a controllable manner. One well known TRP is poly(NiPAAm) which has been established for inducing detachment of cell sheets mainly by the pioneering work of Okano and coworkers.^{6–9}

^{a)}Electronic mail: katja.uhlig@ibmt.fraunhofer.de.

^{b)}Present address: University of Heidelberg, Heidelberg, Germany.

^{c)}Present address: Roche Diagnostics, Rotkreuz, Switzerland.

^{d)}Present address: Institut Charles Sadron, Strasbourg, France.

Recently, a new class of TRP coatings was introduced: poly(MEO₂MA-*co*-OEGMA) is based on poly(ethylene glycol) (PEG).^{10–14} PEG is part of many biomedical and pharmaceutical products due to its remarkable biocompatibility. PEG-coated surfaces are cell-repellent due to (i) a large excluded volume combined with a high degree of hydration, (ii) high PEG chain mobility, and (iii) the low interfacial energy between PEG and water molecules.¹⁵ All these aspects suggest a high functionality of thermoresponsive PEG-based coatings that should manifest themselves particularly in their repellent state. Standard formulations of PEG have LCSTs far above physiological temperatures. But it has been demonstrated that polymers consisting of a hydrophobic backbone and short PEG side groups can be synthesized with LCSTs in a suitable temperature range. Our approach is based on the random copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA). Through a suitable choice of the composition of the comonomers, the LCST of these polymers can be precisely adjusted.^{16–18}

However, our understanding of which factors of the polymer and the layer architecture determine the functionality and efficacy of TRP coatings is still limited, and hence, the more widespread use of these coatings is hampered. A common problem is that layers that allow fast cell adhesion are not particularly effective in inducing cell detachment and vice versa. This means that both states of the polymer above and below the LCST are not fully decoupled. Related to this problem is that TRP coatings of a particular architecture do not perform equally well with cells of different adhesion strengths.¹¹

In order to address these issues, we explore the behavior of L929 mouse fibroblasts on layers of three versions of the TRP poly(MEO₂MA-*co*-OEGMA). These TRPs differ in, first, the length of the hydrophilic polymer backbone which is reported to be a crucial parameter,¹⁹ and second, the composition of the PEG side groups. As a third factor, the molecular density was varied while cell adhesion to and detachment from the TRP coatings was characterized. This is certainly a highly crucial parameter. For identifying an optimal polymer density for the coating, the following considerations may be helpful: On one hand, the coverage should be high enough to ensure sufficient functionality of the coating, especially in the cell-repellent state below the LCST. On the other hand, coatings of very high polymer density do not provide the individual chains with adequate conformational freedom at the transition such that the conformational change at the LCST may be incomplete. In addition, optimal hydration of the polymer in the cell repellent state may not occur, as access of water to densely packed films is impeded. Unfortunately, no reliable concepts of the role of the polymer density in the cell-adhesive state exist. High molecular densities may lead to rather compact coatings that induce strong cell adhesion, but this remains speculative. Finally, it is likely that for both states of the TRP coatings different optimal molecular densities exist. In this work, we present results that help to clarify these open questions.

II. MATERIALS AND METHODS

A. Synthesis of methacrylate derivative-based thermo-responsive polymers with disulfide bonds

The methacrylate derivative-based thermo-responsive polymers were synthesized by atom transfer radical polymerization (ATRP) starting from an initiator incorporating a disulfide bond.²⁰

Copper(I)bromide (CuBr, 95%, Acros, Belgium), 2,2'-bipyridyl, 2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA, 95%, Aldrich, Germany), poly(ethylene glycol)methyl ether methacrylate (Mn = 300, OEGMA 300 or Mn = 475, OEGMA 475, Aldrich, Germany), disulfide initiator, and 2.4 ml of ethanol were added to a Schlenk tube sealed with a rubber septum. The mixture was then degassed under stirring for 15 min by purging with argon. Then, the mixture was stirred in inert atmosphere. The reaction was stopped by exposing the transfer agent to air. The polymers were purified by dialysis against water (ZelluTrans membrane, nominal molar mass cutoff 4–6 kDa, Roth, Germany) and isolated by lyophilization (supplementary Table I).²¹

Copolymer composition was verified by ¹H-NMR spectroscopy²² and found to be in agreement with the monomer feed within the limits of the method.

B. Sample preparation

In order to reproducibly obtain homogeneous coatings, the polymers were chemisorbed to gold surfaces by covalent binding of a central disulfide group. To this end, glass cover slips ($20 \times 20 \text{ mm}^2$, Menzel, Germany) were first coated with chromium (2 nm) and gold (47 nm) using an Auto 500 Electron Beam Evaporation System (Edwards, UK). The freshly prepared gold substrates were immersed into an ethanol solution containing disulphide-poly(MEO₂MA-*co*-OEGMA) with different concentrations for 3 h at room temperature without shaking.¹¹ Unbound polymer was removed by repeated washing of the gold substrate in ethanol for 1 h. Finally, the slides were dried using nitrogen and were immediately used for cell cultivation.

C. Cell experiments

L929 mouse fibroblasts (ACC 2, DSMZ, Germany) were cultivated in Dulbecco's modified eagle medium (DMEM) containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (25 mM), fetal calf serum (FCS) (10%), penicillin/streptomycin (1%), and L-glutamine (2 mM, all Biochrom, Germany) at 37 °C and 5% CO₂. About $10^4 \text{ cells cm}^{-2}$ were seeded on the freshly prepared thermoresponsive poly(MEO₂MA-*co*-OEGMA) surface. The samples were stored in the incubator until the cell spreading time was reached. The cell spreading time is the time from cell seeding until over 50% of the adherent cells changed their morphology from spherical to spread. After this time, the substrates were cooled down to room temperature ($\sim 22^\circ\text{C}$), i.e., across the LCST of the polymer. To observe the morphology for assessing spreading time and rounding efficiency (explained below), the samples were quickly transferred from the incubator to an optical microscope at room temperature equipped with a $10\times/0.25$ objective and a Nikon Digital Sight DS-L1 (Nikon, Germany). After 30 min under microscopic observation, the cells were gently rinsed with a 1 ml pipette. The rounding efficiency is the percentage of cells which changed from the spread to the spherical state.

D. Surface plasmon measurements

Surface plasmon measurements were performed with a Multiscope (Optrel, Germany) and a 60° SF10 glass prism ($30 \text{ mm} \times 30 \text{ mm}$, Linos, Germany) which was mounted on a gold-coated glass coverslip of equal refractive index (SF10, PGO, Germany) by oil immersion (RI: 1.725, Cargille, USA). The incubation chamber was first flushed with ethanol ($n_{\text{medium}} = 1.3614$) and a scan of an uncoated gold surface was performed under various angles of incidence to measure the resonance curve. The optical properties of the sample were analyzed with the software Surface Plasmons Spectroscopy (Optrel, Germany). Then, a kinetic measurement was carried out by using a fixed angle of incidence to detect the light enhancement during adsorption of the TRP after replacement of the fluid (25 μM poly(MEO₂MA-*co*-OEGMA), polymer I). When the light intensity reached a plateau, the chamber was flushed again with ethanol. A new scan of the coated sample revealed the shift of the resonance curve due to adsorption of the TRP. This allows for calculation of the layer thickness (d).

E. Calculation of the surface coverage of molecules

To estimate the surface coverage of poly(MEO₂MA-*co*-OEGMA) molecules at a surface, the increment dn/dc of the refractive index n with concentration c is needed. As an approximation, we employ the increment value of PEG of $0.13 \text{ cm}^3 \text{ g}^{-1}$.^{23,24} Furthermore, the refractive index of the polymer (n_{layer}) was assumed to be 1.5 and the refractive index of the medium (n_{medium}) was 1.3614.

The mass density c is calculated by

$$c = \frac{n_{\text{layer}} - n_{\text{medium}}}{\frac{dn}{dc}} \quad (1)$$

to obtain the mass coverage Γ ,

$$\Gamma = d \cdot c, \quad (2)$$

and from this the molecular density N_{mol} with the Avogadro constant N_A ,

$$N_{mol} = \frac{\Gamma}{\frac{M_w}{N_A}}. \quad (3)$$

F. Measurements of water contact angles

The static contact angles of bare, freshly prepared gold, and poly(MEO₂MA-*co*-OEGMA)-modified gold substrates were measured at 25 °C and 45 °C with a contact angle measuring system G10 (Krüss Surface Science, Germany) including a microscopy heating plate Linkam MS100 (Linkam Scientific Instruments, UK).

III. RESULTS

The cell detachment of L929 mouse fibroblasts from poly(MEO₂MA-*co*-OEGMA)-coated gold substrates upon a decrease of the cultivation temperature from 37 °C to 22 °C has recently been demonstrated.^{10,11} In these reports, we also analyzed cell spreading: the time from cell seeding until spreading was considerably longer (several days) than on conventional cell culture substrates (several hours). To overcome this, in this report, we investigate the effect of (i) the molecular structure and (ii) the concentration of the coating solution during surface preparation of thermoresponsive polymer surfaces.

Concerning the first factor, the table in Fig. 1 provides information on the properties of three different poly(MEO₂MA-*co*-OEGMA) polymers. Two main aspects were in the center of the analyses here: first, the influence of the degree of polymerization (DP , number of monomers per polymer unit), and second, the influence of the side chain length of OEGMA (number of monomers z) on cell adhesion and detachment. Additionally, the ratio of MEO₂MA (x) and OEGMA (y) were varied. Therefore, the different polymers **I** ($DP = 57$; $z = 9$), **II** ($DP = 44$; $z = 9$), and **III** ($DP = 58$; $z = 4$) were synthesized. The composition of the side chains determines the LCST of the three polymers which was measured by turbidimetry and is also given

FIG. 1. Chemical structure of the three poly(MEO₂MA-*co*-OEGMA)-based TRP which differs in their ratio of MEO₂MA (x) and OEGMA (y) and the chain length (z) of the OEGMA side chain. The table also gives the resulting LCST measured by turbidimetry and the molecular weight determined by gel permeation chromatography.

in Fig. 1. The LCST of the polymer in solution derived by turbidimetry¹⁰ is in good agreement with the LCST of the polymer at the surface measured by adhesion force as a function of temperature.²⁵

To identify the impact of the chemical composition of the polymers on cell adhesion, the cell morphology of L929 mouse fibroblasts on different TRP-coated substrates and on a control substrate (bare gold) was observed as a function of cultivation duration (Fig. 2). All of these thermoresponsive polymer substrates were produced with the same concentration of the coating solution ($5\text{ }\mu\text{M}$). The spreading of cells on polymer **I**-coated surfaces took longest. For the first 3 days, the cells grew in clusters which became smaller with progressing time. After 4 days of cultivation, the cells started to move outside the clusters and developed lamellipodia. Five days after cell seeding, over 50% of the cells were spread on the surface. On polymer **II**-modified substrates, the cells took 3 days and on polymer **III**-modified substrates 2 days for cell spreading. In contrast, the control substrate (gold) mediated cell spreading within 1 day.

To examine the impact of the second factor, the polymer surface density, on the cellular behavior, the concentration of the coating solution was changed. Instead of varying the adsorption time, we have chosen to vary the concentration of the solution, because in the latter approach the adsorption process reaches thermodynamic equilibrium and, hence, yields reproducible results. Kinetic control over the film formation process is difficult to achieve, since the rate constants for the various steps of film formation, such as the initial physisorption, chemisorption, and eventual healing of the film, are difficult to estimate.²⁶ In addition, film formation is at least biphasic.²⁷ The relevant functional changes occur at high surface densities when the second, slow process dominates and where it is difficult to identify criteria for the termination of the film formation before a steady state is reached. Fig. 3(a) shows the adhesion behavior of L929 mouse fibroblasts as a function of the polymer density. The cells show a similar tendency on all three polymers in dependence of the concentration of the coating solution. On polymer **I**- and **II**-modified substrates, spreading times (for definition, see Sec. II) increase with increasing concentration to a maximum at a range between $5\text{ }\mu\text{M}$ and $10\text{ }\mu\text{M}$. In accordance with the results presented in Fig. 2, the spreading time of cells on polymer **I**-modified substrates was about 2 days longer than on polymer **II**. On polymer **III**-coated substrates, the cell spreading time monotonically increased with increasing concentration, so no maximum in the spreading

FIG. 2. Phase contrast images of L929 mouse fibroblasts on different poly(MEO₂MA-*co*-OEGMA)-coated gold substrates and uncoated gold substrates after different cultivation durations. The time needed for complete cell spreading after seeding and subsequent sedimentation of spherical cells from suspension strongly depends on the polymer. The concentration of the coating solution was $5\text{ }\mu\text{M}$ in each case. The scale bar is $100\text{ }\mu\text{m}$.

FIG. 3. Spreading time (a) and rounding efficiency (b) of L929 mouse fibroblasts on substrates coated with different poly(MEO₂MA-co-OEGMA) polymers as a function of the polymer concentration of the coating solution. The lines only serve as guides to the eye.

time could be observed. At 100 μM , the highest concentration used, no cell spreading was observed within 6 days.

After investigation of the spreading time, we studied the dependence of the rounding efficiency on both factors, i.e., polymer structure and coating density (Fig. 3(b)). The cells were cultivated on the different surfaces until they were spread. Afterwards, the samples were cooled down from cell culture temperature to room temperature for 30 min, and the morphological changes were documented to analyze the rounding efficiency (Fig. 4). Cells on all three polymer-coated substrates show a very high rounding efficiency of about 90% generated from a polymer concentration of 1 μM –50 μM . In the case of polymer I, this also holds for 100 μM . All of these surfaces also allow for a complete cell detachment by rinsing. At the highest concentration of 100 μM , L929 mouse fibroblasts on polymer II surfaces round up with an efficiency of only $19\% \pm 5\%$, which is similar to the rounding efficiency of cells on pure gold substrates ($15\% \pm 9\%$). Cells can also not be detached by rinsing in these two cases. On coatings prepared from 100 μM polymer III solutions, no cell spreading could be observed.

For further surface characterization, contact angles of water were measured below and above the LCST for the different poly(MEO₂MA-co-OEGMA)-modified substrates. Contact angles indicate the hydrophilicity and the macroscopic homogeneity of a surface. In several

FIG. 4. Optical phase contrast images of L929 mouse fibroblasts on TRP-coated gold substrates (a)–(c) and on pure gold substrates (d)–(f) were taken at 37 °C (a) and (d) and after being exposed to room temperature (~ 22 °C) for 30 min (b) and (e). After this treatment, the cells could easily be removed by rinsing (c) and (f). The scale bars are 100 μm .

investigations, substantial changes of the contact angle on poly(NiPAAm)-coated substrates were reported when the temperature passes the LCST.^{6,28} In our systems, we do not observe such changes. At 25 °C, the contact angles of the three different polymer-modified substrates were equal. The contact angles were in the range of $54.3^\circ \pm 0.5^\circ$ for polymer **I**, $55^\circ \pm 1^\circ$ for **II**, and $56.8^\circ \pm 0.8^\circ$ for **III**. After heating the surfaces to 45 °C, the contact angles did not change significantly (I: $55.0^\circ \pm 0.5^\circ$, II: $55.5^\circ \pm 0.6^\circ$, and III: $57.0^\circ \pm 0.8^\circ$). The small variance of the contact angles at different positions on each surface indicates that they were macroscopically homogenous. Additionally, we compared adhesion forces of the polymer layers below and above the LCST using the colloidal probe technique. In contrast to the contact angle measurements, these force experiments showed two clearly distinct adhesion force regimes.²⁵

For determining surface coverage, surface plasmon resonance (SPR) was employed. Here, we present the measurement of the formation of a layer from a 25 μ M polymer solution of polymer **I**. For this purpose, the resonance angle was first measured for a bare gold substrate (curve 1 in Fig. 5(a)). Next, the kinetics of the immobilization was followed by recording the reflectivity at a fixed angle at the steepest part of the resonance curve (Fig. 5(b)): after application of polymer **I**, the reflectivity quickly increased and went into saturation after 2 h. After rinsing, the reflectivity decreased (by about 0.6). Finally, a second resonance curve was recorded with the complete coating (curve 2 in Fig. 5(a)). The resonance angle θ between both readings had shifted by about 0.8° , corresponding to a layer thickness of 4.5 nm ($n = 1.5$). This value can be used for calculating the molecular density ($0.23 \text{ molecules nm}^{-2}$). One molecule of polymer **I**, therefore, occupies an area of 4.3 nm^2 . Assuming a length of the polymer backbone of 18 nm neglecting the side chains, we calculated a radius of gyration that corresponds to a molecular surface area of the polymer of approximately 2 nm^2 .

In addition, the long-term stability of polymer **I**-modified substrates under two different conditions was tested (data not shown). The samples were stored for 6 months either at room temperature or at 45 °C. The samples kept at room temperature maintained their functionality (rounding efficiency). In contrast, for samples stored above the LCST, the rounding efficiency decreased dramatically after 10 weeks to values indistinguishable from those of control substrates, i.e., around 15%.

IV. DISCUSSION

Applying enzyme cocktails containing trypsin for the detachment of adherent cells from their cultivation substrates is unacceptable for many protocols. TRP coating represents a promising tool for avoiding such a harsh treatment of cells. However, the applicability of these coatings is still limited, as the relation between their structural details, and their function is by far not fully understood. In an effort to improve this, we investigated the interaction of three

FIG. 5. (a) Measurement of the surface plasmon resonance on a blank gold substrate (black) and after immobilization of 25 μ M polymer **I** (gray). The shift of the resonance angle $\Delta\theta$ of 0.8° corresponds to a layer thickness of 4.5 nm using a refractive index of 1.5. (b) Kinetic immobilization measurements of polymer **I** on a gold substrate at a constant angle (55.5°). After 2 h, the reflectivity signal goes into saturation. For details see text.

different polymers that are based on poly(MEO₂MA-*co*-OMEGA) and that are covalently anchored to gold substrates to L929 mouse fibroblasts. This approach provides us with the possibility to systematically alter factors that we assume to be crucial for the performance of these coatings. The structural flexibility of the polymer enables us to vary the composition of the highly biocompatible side groups, their length, and the length of the polymer backbone with relative ease. Utilizing sulfur-gold-chemistry allows us to systematically and reproducibly alter the polymer density by controlling the concentration of the polymer solution. As parameters for the evaluation of the response of the cells, we determined (i) the time the cells require to adopt a morphology that is indicative for the formation of a sufficient degree of adhesion and (ii) the percentage of cells that assume a spherical morphology after having been exposed to a temperature below the LCST for 30 min. Both parameters are most crucial for the evaluation of the suitability of thermoresponsive coatings for cell culturing applications.

The challenge for achieving an optimal performance of these coatings is to induce swift cell adhesion to the substrate above the LCST and efficient and fast detachment of as many as possible cellular adhesion domains when the temperature is decreased below the LCST. In general, the measures for optimizing one parameter, e.g., the adhesiveness of the coating through the introduction of suitable molecular groups, easily affect the efficiency of the coating to induce cell detachment below the LCST. As the options for molecular rearrangement in such ultrathin layers are finite, the accessibility of molecular groups that are essential for the functionality of one state of the coating can hardly be completely prevented in the other state. In this context, the polymer density in the layer is an important parameter. At very low coverage of the polymer, the properties of the support are not sufficiently screened and, hence, dominate the interaction between the cells and the substrate. The cellular adhesion is then controlled by the surface properties of the support. At very high surface densities of the polymer, the configurational space of the macromolecule may be reduced such that it cannot sufficiently switch its conformation at the LCST. In addition, the model of steric repulsion for PEG brushes introduced by Andrade and de Gennes provides insight, although it is derived for layers formed from polymer brushes.²⁹ It predicts a strong dependence of protein adsorption to coatings of these polymers on their surface density. Hence, we would have expected the variation of surface density of the thermoresponsive polymer to play a prominent role in its repulsive state below the LCST when it is assumed to be highly hydrated. Although it is not clear to what extent ECM proteins secreted by the cells mediate their adhesion to synthetic substrates, steric repulsion may also globally affect cell adhesion. In the cell-adhesive state, the intramolecular interactions are supposed to dominate, as the polymer assumes a collapsed coil structure. Hence, the strong sensitivity of the cell-adhesive state of the coating to the molecular density is surprising. Our SPR data, which give a quantitative measure of the surface density of the tethered polymer, together with data on the mass density of PEG brushes²⁹ allow us to propose a simple box model of the polymer that may explain the behavior of the polymer coating below and above the LCST. A more detailed analysis of the grafting density of layers as a function of the polymer concentration in solution using SPR was not performed, since in our set-up the resolution of this method is not sufficient to resolve the small changes in coverage that are responsible for the changes in cell behavior observed. From our measurements, an area per chain of polymer I of 4.3 nm² is derived (the molecule consists of two chains that are linked by the disulfide group). This is about twice the area estimated from the radius of gyration of the polymer backbone when neglecting the spatial requirement of the side chains, which represent approximately 70% of the molecular weight of the polymer. Taking a typical value of 0.21 g cm⁻³ for the mass density of PEG brushes tethered to a surface,²⁹ we calculate that the polymer extends approximately 23 nm away from the surface. If we assume a mass density of 0.7 g cm⁻³, which is representative for an ordered lipid phase,³⁰ the polymer dimension along the surface normal corresponds to 6.9 nm. Given the approximate length of the polymer backbone in a fully stretched configuration of 15 nm, we conclude that the mass density of our thermoresponsive coating is considerably higher than that measured in pure PEG layers in a brush-like architecture. In this context, it is worth mentioning that each monomer unit carries a side group with considerable spatial requirements. This may lock the polymer backbone in an

extended configuration. Based on these considerations, the polymer chain may be described by a box model with a $2.1 \text{ nm} \times 2.1 \text{ nm}$ base area and a greater height for all reasonable values of the mass density of the thermoresponsive coating. This suggests that, in the film, the polymers assume a brush-like arrangement. Due to the lateral packing constraints imposed by the side groups, only the extension into the medium and the compactness may vary, while the orientation of the backbone is maintained upon switching between both states. In this respect, the absence of changes in the contact angle above and below the LCST was to be expected. Taken these considerations together, the molecular organization of the polymers in the coating may be more adaptive to the cell-repellent state than to the cell-adhesive state above the LCST. The maximum of the spreading time as a function of the polymer density that we observe may then be the result of a molecular arrangement where the ethylene glycol side groups are exposed such that they possess sufficient degrees of configurational freedom and are well hydrated. Increasing the polymer density beyond the value that yields maximum spreading time may then reduce the hydration of the side chains.

Interestingly, the rounding efficiency is hardly affected by the polymer densities investigated. This may suggest that the spatial requirements of the polymer configuration above the LCST determine the packing of the molecules. Hence, the more extended polymer configuration below the LCST allows for sufficient space such that a high degree of hydration and flexibility is possible for all densities tested. Only, for the layers produced from $100 \mu\text{M}$ polymer **II**, the cell-repellent character of the layer is lost.

Whether the behavior of the layers formed from the polymer **III** as a function of surface density is qualitatively different from the other two polymers cannot be answered at this stage. First, identical concentration values of the solution for the various polymers do not necessarily lead to comparable surface densities, as the molecular details of the respective polymers have a large impact on the film assembly. Second, a maximum of the spreading time for the polymer **III** could not be observed, as cell adhesion at coatings formed from self-assembly solutions above a concentration of $50 \mu\text{M}$ did not occur within reasonable time limits.

Three structural variants of the PEG-based thermoresponsive polymer have been synthesized, and the functionality of the corresponding coatings has been studied. The decision to test two polymers of different molecular weights (I and II) and with an identical composition of the side groups was initially motivated to better understand how the length of the polymer affects the structural transition at the LCST as it largely influences the intramolecular degrees of freedom. All three polymers show a comparable rounding efficiency in the concentration range tested. Above the LCST, the ability of the thermoresponsive coatings to induce cell adhesion appears to be more sensitive to the molecular structure of the polymers. This implies that our box model may be applicable to all three polymers. The two polymers with an almost identical composition of the side groups (I and II) show a similar qualitative behavior. However, cells adhere considerably faster to layers formed from the shorter polymer **II**, assuming that identical concentrations of the two polymer solutions lead to comparable surface densities. This can be rationalized within the model for steric repulsion of PEG coatings.²⁹ Longer polymer chains possess additional degrees of configurational freedom and give rise to a higher osmotic pressure. The composition of the PEG side groups of the polymer **III** is such that the LCST is considerably lower than those of the two other polymers. Hence, for this polymer, the cell cultivation temperature of 37°C is more distant from the LCST than for the other two polymers. Given the width of the transition of these polymers of at least 6 K ,³¹ the polymer **III** is expected to be considerably less hydrated at 37°C . As a consequence, the shorter spreading times measured on the polymer **III** may indicate that at 37°C it is in a robust cell-adherent state, whereas the two other polymers are still in an intermediate, more hydrated state at this temperature. This could mean that the steric repulsion power per individual polymer chain is lower for the polymer **III** than for the two other polymers and, hence, a higher surface density is required to reach the maximum spreading time. Unfortunately, at these higher densities, no cell adhesion could be observed within reasonable times.

In summary, for further improvement of the functionality of PEG-based thermoresponsive coatings, measures should focus on the cell-adhesive state for reducing the time that cells

require to properly spread and eventually proliferate. For such an approach, compromises may be acceptable that potentially have negative effects on the ability of the coatings to repel cells, as this property is more robust. The parameters that should be investigated in more detail are the chain length and the composition of the PEG side groups, which defines the LCST, as the effects of variations of these parameters appear to be reasonably predictable. Eventually, introducing molecular groups such as short peptides that enhance cell adhesion into the polymer or as individual units into the coating may have great potential. However, to what extent the incorporation of these groups is beneficial to the functionality of the coatings depends very much on their precise location and their dynamics during the transition. Hence, molecular dynamics calculations would be a rewarding approach for elucidating the structural and functional details of these polymer systems in order to improve their performance.

V. CONCLUSION

In this work, we have shown that cell adhesion can successfully be controlled using the PEG-based thermoresponsive polymer poly(MEO₂MA-*co*-OEGMA). Variations of the molecular structure and the polymer density of the coatings had a considerable impact on the time required for sufficient cell spreading and, less pronounced, on the efficiency of cell rounding. While the latter is sufficient for a regular utilization of these systems for cell cultivation, still some work has to be invested to shorten the spreading time in order to improve the functionality of these coatings. Although we suggest various measures for such an improvement, we believe that future molecular dynamics calculations for modeling PEG-based thermoresponsive coatings are crucial, as they would provide us with a rational approach that may overcome present limitations of these systems.

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