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Historical perspective

Surface-bound microgels — From physicochemical properties to biomedical applications

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ABSTRACT

Microgels offer robust and facile approaches for surface modification, as well as opportunities to introduce biological functionality by loading such structures with bioactive agents, e.g., in the context of drug delivery, functional biomaterials, and biosensors. As such, they provide a versatile approach for the design of surfaces with pre-determined characteristics compared to more elaborate bottom-up approaches, such as layer-by-layer deposition and surface-initiated polymerization. In the present overview, properties of surface-bound microgels are discussed, ranging from physical adsorption and covalent grafting in dilute systems, to directed self-assembly, multilayer structures, and composites, as well as loading an release of drugs and other cargo molecules into/from such systems, and biomedical applications of these.

Contents

1. Introduction

Microgels are sparsely cross-linked colloidal gel particles, frequently displaying dramatic swelling transitions in response to one or several of a wide range of stimuli. Depending on the characteristics of microgel monomers and other incorporated compounds or nanoparticles, microgels can be designed to be environmentally responsive to a number of key triggers, e.g., changes in temperature, pH, ionic strength, redox conditions, or specific metabolites [1–3]. They can also be designed to display triggerability controlled by external fields, such as light/near-infrared, or magnetic fields. Due to this, and their frequently fast response time, microgels have attracted interest in a variety of fields [4–8]. While the majority of microgel research has so far concerned microgel suspensions and their applications, there is increasing interest also in surface-bound microgels. Here, surface modification approaches

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range from physisorption, with and without covalent attachment, to spin-casting, wrinkling, and other strategies for induced assembly [9–11]. For structures with increasing complexity, microgel deposition can also be combined with layer-by-layer deposition to multilayer structures, or with secondary cross-linking to incorporate individual microgel particles into a matrix. Most of these offer robust and facile approaches for surface modification and functionalization, and opportunities to design biological functionality. For example, depending on microgel properties and interfacial structure, serum protein adsorption can be reduced, as can non-specific bacterial and cell adhesion, and adverse biological response, such as generation of pro-inflammatory cytokines. Furthermore, by loading such structures with drugs and other bioactive agents, biological response can be obtained related to triggerable release or to surface-localized response caused by covalently immobilized drugs, the exposure of which again triggerable due to the responsive microgel particles [4–6]. Through this, opportunities are offered in localized drug delivery e.g., in the context of depot formulations and focal therapies, but also in functional biomaterials and regenerative medicine, in which the active agent can be, e.g., a substance triggering differentiation of stem cells or cell proliferation for promoted tissue integration. Furthermore, due to their large-scale swelling/deswelling transitions, opportunities are offered for microgel-based biosensors, either on their own or in combination with inorganic (notably metal) nanostructures for interference-based and related signal detection [8].

In the present overview, the current understanding of surface-bound microgels is briefly outlined, focusing on factors affecting structure and responsiveness of both individual surface-bound microgels and microgel surface assemblies of varying complexity. In addition, the application of such systems for loading and release of drugs and other cargo molecules is illustrated, with particular focus on drug delivery, functional biomaterials, and biosensors.

2. Adsorbed and covalently bound microgels

2.1. Nonionic microgels

Due to their abrupt and dramatic swelling/deswelling in response to relatively minor temperature changes, poly(N-isopropylacrylamide) (PNIPAM)-based microgels have received considerable interest as surface coatings. Although such temperature-responsiveness can be obtained also by other types of surface coatings, such as adsorbed or covalently bound linear PNIPAM-based polymers, microgel-based surface coatings generally offer more dramatic effects due to the larger swelling/deswelling transition obtained, caused by the connectivity within the microgel particles. For example, Burmistrova et al. compared thermosensitive surface coatings formed by PNIPAM microgel adsorption to those formed by PNIPAM-based polyelectrolyte multilayers containing a diblock copolymer with a large PNIPAM block. While the multilayers showed a small and irreversible response to an increased temperature and reduced solvency, the adsorbed microgels showed a pronounced and reversible response [11]. Characterizing the temperature-dependent swelling/deswelling of PNIPAM-based microgels further, Höfl et al. investigated the swelling behavior of poly(N-isopropylacrylamide-*co*-vinylacetic) acid microgels using dynamic light scattering, neutron scattering, and AFM at various vinylacetic acid content. Microgel particles adsorbed onto glass substrates were found to display a similar temperature-induced volume phase transition as their counterparts in suspension, but with their swelling capacity reduced by one order of magnitude compared to the equivalent microgel suspensions. From comparison between microgels in suspension and individual adsorbed microgels, it was also concluded that the continuous character of the transition observed in solution does not arise from particle polydispersity, but can instead be attributed to the heterogeneity inside each individual microgel particle (Fig. 1) [12]. Also comparing solvency-induced deswelling of PNIPAM-based microgels in suspension to that of surface bound microgels, Richter et al. investigated the effect of solid interfaces on the co-nonsolvency effect for PNIPAM-based microgels containing different contents of allyl acetic acid, using light scattering and AFM for mixtures of water and organic solvent (tetrahydrofuran, ethanol, and isopropanol). For microgels deposited as silicon wafers pre-coated with poly(allylamine hydrochloride) (PAH), the minimum in particle volume due to co-nonsolvency was found to display a pronounced shift, from 10–20% of organic solvent to 40–50%, after deposition at the Si/PAH wafer, indicating a substantially higher water to organic content within the surface-bound microgels compared to microgels in solution [13].

Investigating how the mechanical properties of surface-bound PNIPAM-based microgels depend on the temperature-dependent swelling/deswelling transition, Tagit et al. investigated PNIPAM microgels synthesized by surfactant-free radical polymerization, probing morphology and nanomechanical properties of single PNIPAM microgel particles at the silicon/air and silicon/water interfaces by AFM [14]. The elastic modulus thus obtained showed the modulus of the surface-bound PNIPAM microgels to increase by one order of magnitude when crossing the volume phase transition temperature (VPTT), from the swollen to the collapsed states, reflecting a more compact chain packing within the microgels. Similarly, Hashmi and Dufresne reported fully collapsed PNIPAM microgels to be about ten-fold stiffer than those in the fully swollen ones (elastic modulus 123 and 13 kPa, respectively) [15]. The magnitude of the collapse/stiffening seems to vary quantitatively between systems, however. Illustrating this, Matzelle et al. investigated mechanical properties of PNIPAM gel surfaces by scanning force microscopy in air or in water below and above the phase transition, using both spherical (micrometer sized) and conical probes. For the swollen state at 10 °C (<<VPTT), Young's modulus was found to be >100 times lower than for the collapsed state at 35 °C (>VPTT) [16].

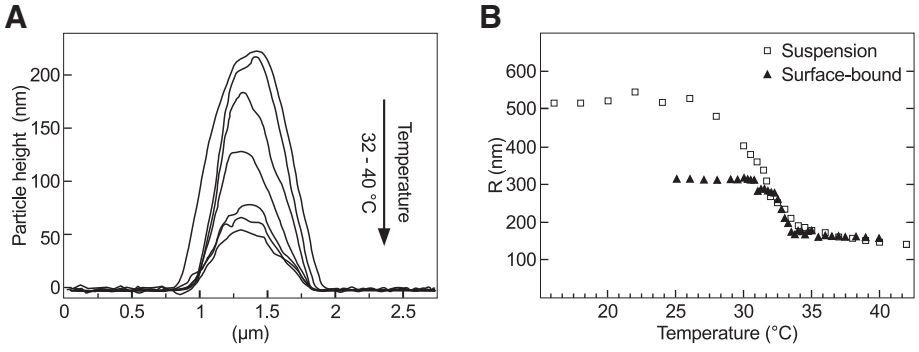

Fig. 1. (a) Temperature-dependent deswelling, in water, of PNIPAM-VA microgels (VA content 1 mol%) adsorbed to glass substrates. (b) Comparison of temperature-dependent swelling of PNIPAM-VA microgels (VA content 1 mol%) in microgel suspension and after adsorption at glass substrates. As can be seen, microgel swelling at low temperature is substantially suppressed after microgel adsorption.
(Re-drawn from [12].)

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As with microgels in suspension, the magnitude of the swelling/deswelling transition of surface-bound microgels, as well as the mechanical consequences of such transitions, depend on various factors, such as the microgel composition and cross-linking density. Addressing the latter, albeit for macrogels rather than for adsorbed microgels, Matzelle et al. investigated PNIPAM and poly(acrylamide) (PAAm) hydrogels of varying cross-linker concentration. For this, both spherical micron-sized probes and sharp conical probes were used for determination of Young's moduli. Again, a dramatic increase of the stiffness (up to 10 times) was observed when crossing the phase transition temperature. Furthermore, the cross-linker concentration was shown to strongly influence the Young's modulus, particularly for temperatures above the critical temperature, while only small variations were seen for temperatures below the transition [17]. Similarly, the moduli of PAAm hydrogels were found to increase strongly with increasing cross-linker concentration. For microgels containing a cross-linking gradient, Fernandes et al. reported on an approach to model the dependence of the volume and elastic modulus, in which the inhomogeneous polymer gel is divided into an arbitrary number of independent and homogeneous layers, each treated independently by the Flory-Rehner approach. AFM measurements of volume and elastic modulus of PNIPAM microgels deposited on a poly(ethyleneimine)-coated glass were found to be in good agreement with the model, assuming the gel has a harder core and softens towards the outer shells [18].

Analogous to effects of cross-linking, degradation may affect structure and mechanical properties of surface-bound microgels. Investigating such effects, Richter et al. employed AFM for real-time visualization of the pH-responsive degradation of polyglycerol-based nanogels containing acid-labile acetal bonds (Fig. 2) [19]. During degradation, the particle height was found to decrease exponentially as a function of time, whereas the particle width increases, particularly during early stages of particle erosion. Furthermore, the results indicate that particles effectively undergo “de-cross-linking” into their starting macromonomers on degradation. While the lighter cross-linker dissolves into the solution during this process, the heavier polyglycerol-compartments remain at the surface. Using AFM, South and Lyon similarly investigated microgel degradation of poly(N-isopropylmethacrylamide-*co*-acrylic acid), cross-linked with a hydrolyzable cross-linker [20]. In doing so, the glass substrates used for microgel deposition were removed from the degradation solution after different degradation times, monitoring the resulting microgel films by AFM. Initially, microgel swelling was found to increase due to the hydrolysis of the cross-linker and the concomitant decrease in network connectivity. After longer erosion times and extensive polymer loss, the remaining degraded microgel particles are characterized by a dense center and a low-density corona.

In addition to the cross-linking density, the structure and mechanical properties of PNIPAM-based and other surface-bound microgels can be controlled also by other means. For example, investigating the effects of interfacial layer composition, Sorrell and Lyon reported on the assembly of two-component PNIPAM-based microgel monolayers onto glass substrates via physisorption [21]. By using two microgels of nearly identical size but different softness, the influence of particle deformation on film composition could be determined. In doing so, microgel properties obtained by light scattering were used to predict film composition as a function of solution composition using a random sequential adsorption model. Deviations from the predicted particle adsorption were subsequently taken to be due to particle deformation on adsorption. In addition, mixtures of degradable and non-degradable core/shell particles were used, allowing particle identification after erosion of the microgel cores. Together, the results obtained show that the competition for surface adsorption from binary microgel mixtures is largely determined by the interactions of the components with the surface and their subsequent deformation (Fig. 3). Also investigating two-component microgel mixtures, Saxena and Lyon reported on the influence of microgel packing on colloidal-phase mediated heteroaggregation, using aminobenzophenone-functionalized polystyrene core particles together with poly(N-isopropylacrylamide) and poly(N-isopropylmethacrylamide) microgels of varying cross-linking density [22]. Through this, investigation of the effect of softness on microgel deformation at curved interfaces are facilitated by eliminating the need for electrostatic surface binding. Microgel deformation and swelling were characterized by AFM and viscometry, whereas SEM and pore translocation experiments were used to determine the microgel coverage on the resulting raspberry-like particles (RLPs). Based on this, microgel composition was found to strongly influence the microgel coverage of such RLPs, in turn related to the degree of microgel deformation at the interface. Saxena and Lyon also investigated the influence of microgel composition on the phase behavior of these binary microgel dispersions, comparing the dispersion phase behavior at a planar interface to that at curved interfaces in the form of raspberry-like patchy particles [23]. Again, microgel composition was found to have a considerable effect on the ability of binary dispersions to coat planar and curved interfaces. In particular, binary microgel dispersions containing higher cross-linker content were found to display decreased packing densities, particularly at curved interfaces.

Mirroring the dramatically increased interest in particle-stabilized (Pickering) emulsions and foams during the last decade or so, adsorption of microgels to liquid-liquid and liquid-gas interfaces has also attracted attention in the search for ways to achieve stimuli responsiveness of such emulsions and foams. Due to the dynamic nature of both the interface and the microgel particles, adsorption is more complex than that for solid-liquid interfaces, and adsorption dynamics of the soft microgel particles key for the design of stimuli-responsive stabilizers of Pickering emulsions and foams. It is generally found that emulsification leads to considerable microgel flattening, depending on the

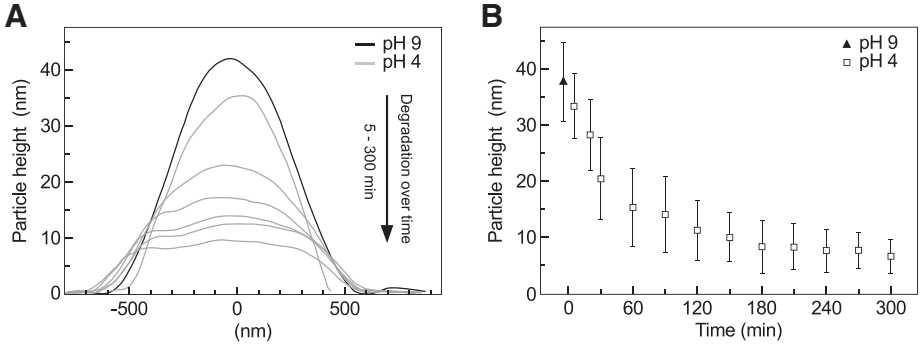

Fig. 2. Effect of chemical degradation on the structure of polyglycerol-based microgels after binding to gold-coated silicon substrates. Shown are cross-sections of a representative microgel particle (a) and average microgel heights (b) after varying times of acid-induced hydrolysis of acetal bonds within the microgels. For comparison, data obtained at pH 9 (i.e., before degradation) are included as well.
(Re-drawn from [19].)

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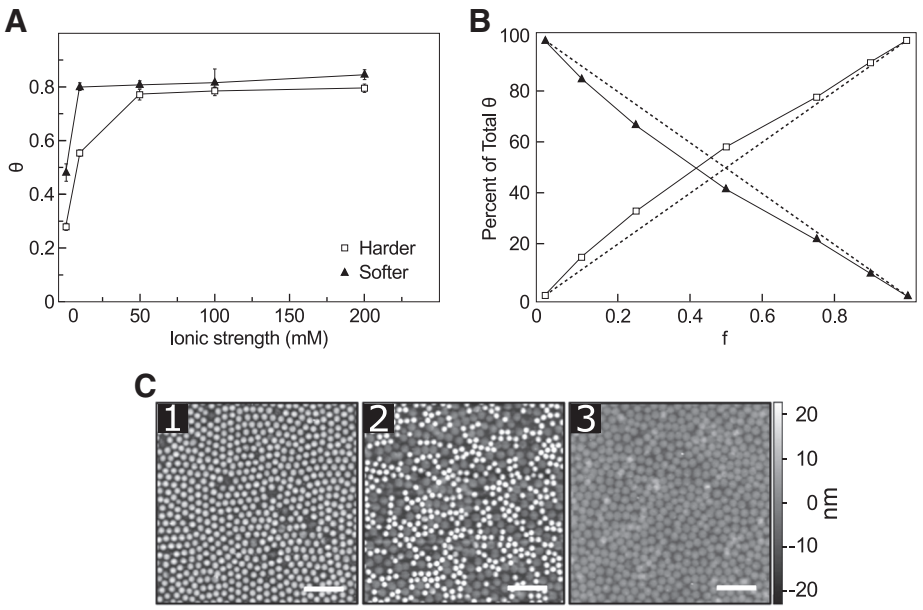

Fig. 3. Microgel deformation influences surface layer composition in mixed microgel systems. (a) Mixed systems of PNIPAM/bis(acrylamide) and PNIPAM/dihydroxyethylene-bis(acrylamide) microgels (both carrying a PNIPAM-AAc shell) form surface coatings at glass substrates which are underrepresented in the latter, more deformable, microgels compared to the ideal random sequential adsorption case. (b) This is due to the larger deformation of the softer microgels at the surface, illustrated also by the larger limiting surface coverage for a similar number of microgel particles. (Re-drawn from [21].) (c) Representative AFM images of binary microgel monolayers in ratios of (1) 1:0, (2) 1:1 and (3) 0:1, scale bars 5 μM.
(Adapted with permission from [21]. Copyright 2008 American Chemical Society.)

balance between maximizing the contact between the interface and the microgel, on one hand, and particle elasticity, on the other [24–29]. In the ideal case, the extent of deformation is given by $\Delta\gamma/\varepsilon$, where ε is the Young's modulus of the microgel particles and $\Delta\gamma$ the interfacial tension. For microgels at the air–water interface, this gives deformations of ≈ 1 μm, which are comparable to, or larger than, the size of the microgel particles. The interfacial deformation, in turn, is of importance for microgel packing at the interface, not the least from a kinetic perspective. As both emulsification and foam generation rely heavily of adsorption kinetics, these aspects ultimately play key roles for determining the stability and other properties of microgel-stabilized emulsions and foams. Addressing these effects, Deshmukh et al. investigated the adsorption of PNIPAM microgel particles at the air-water interface using the Langmuir film balance. The equation of state was determined in equilibrium measurements, as was the adsorbed amount as a function of time. It was found that microgel adsorption at the air-water interface followed mixed-kinetic adsorption, which is initially controlled by the diffusion of particles towards the interface, followed by a slow exponential relaxation, indicating the presence of a coverage-dependent adsorption barrier related to particle crowding at the interface [25]. Similarly, Pinaud et al. investigated microgel adsorption at model oil-water interfaces, as well as microgel deformation on compression and effects on microgel packing and surface elasticity [24]. The structure of the film under forced compression was monitored using Langmuir films and compared to that obtained under spontaneous adsorption using the pendant drop method. The behavior of microgels was found to differ significantly from that of non-deformable particles, but to resemble that of deformable macromolecules, such as linear polymers or soft proteins. Based on this, an approach was proposed for controlling microgel surface packing and resulting flow properties of microgel-stabilized emulsions. Again, interfacial microgel deformation is expected to depend on their "softness", in turn depending on, e.g., cross-linking density or the degree of swelling. Addressing the latter, Kwok and Ngai investigated PNIPAM microgel-stabilized Pickering emulsions. Employing confocal laser scanning microscopy to examine the structure of soft PNIPAM-based microgel particles at the decane-water interface in a microgel-stabilized emulsion, these authors demonstrated that while microgel deformation was not significant in the collapsed state, swollen microgel particles exhibited anisotropic deformation [30].

2.2. Charged microgels

Investigating electrostatic swelling transitions of surface-bound microgels, Nyström et al. studied of poly(ethyl acrylate-*co*-methacrylic acid) microgels, covalently bound to silica surfaces [31]. After surface immobilization, microgel swelling was found to be anisotropically hindered and the structure flattened, to an extent dictated by pH and microgel composition. Microgel deformation under applied load was shown to depend on microgel charge density, with highest deformations at intermediate charge densities. Two modes of microgel deformation under load was observed, one elastic and one viscoelastic, related to polymer strand deformation and displacement of trapped water, respectively. Furthermore, results on polymer strand dynamics showed that the microgels are highly dynamic, as the number of strand-tip interaction points displayed a 4-fold increase during a 10 s contact time. Finite element modeling was able to capture these effects qualitatively, showing also that stress propagation in microgel network decays locally at the rim of contact with a solid interface or close to an AFM tip probe, respectively (Fig. 4) [31].

Somewhat related, Howard et al. investigated pH-dependent swelling/deswelling of poly[2-(diethylamino)ethyl methacrylate] microgels adsorbed to silica surfaces by optical reflectometry and quartz crystal microbalance [32]. Apart from determining the amount and the thickness of the adsorbed microgel in both the swollen and deswollen state, studies of the transition between these showed microgel deswelling to be a slower than swelling. The latter was inferred to be due to the formation of a dense outer layer or skin during collapse, which slows down the deswelling process. Furthermore, surface attachment substantially hinders the swelling capacity of the microgel film. Thus, in its swollen state, the film was found to be only 3–4 times thicker than the collapsed film, whereas for microgel particles in suspension, the volume increases by a factor of 20 upon protonation of the tertiary amine residues. As a consequence of this, even the collapsed film

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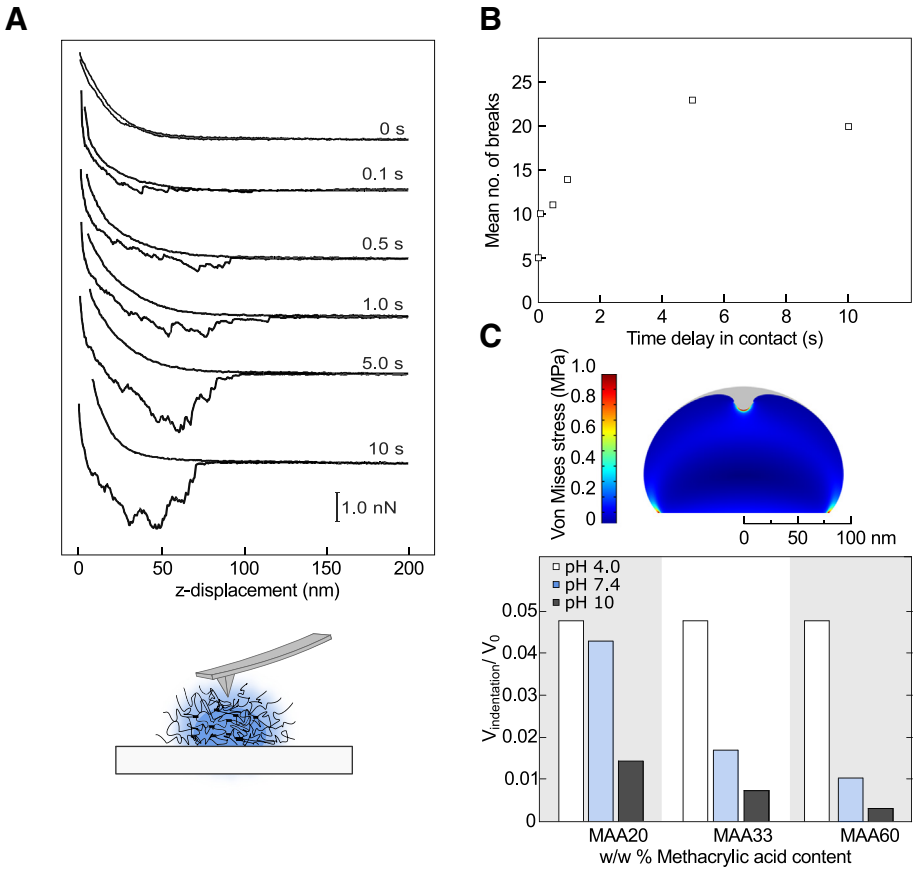

Fig. 4. Chain mobility within surface-bound microgels can be high for swollen microgel systems. (a) Representative AFM force-distance curves for silicon nitride tip interaction with silica-bound poly(ethyl acrylate-*co*-methacrylic acid) (33 mol% methacrylic acid) microgels at pH 7.4 and 10 mM ionic strength. With increasing tip-microgel interactions, the number of chain attachments with the AFM tip increases strongly (b), despite the small relative volume probed by these measurements (c). (Re-drawn from [31].)

contains a considerable amount of water, and the viscoelasticity of the deswollen film is similar to that of the swollen film, suggesting the degree of cross-linking to be the primary determinant of viscoelasticity in this system.

Furthermore, FitzGerald et al. investigated structural changes of pH-responsive microgel particles at silica and mica surfaces. In doing so, lightly cross-linked poly(2-vinylpyridine) (P2VP) particles were first adsorbed in their non-solvated latex form at pH 4.8 to form a disordered monolayer [33]. Reducing pH caused microgel protonation and resulted in partial desorption, as well as in substantial microgel swelling, resulting in the formation of a uniform, swollen film with localized hexagonal packing. Increasing pH again resulted in partial deswelling, as well as in the formation of individual oblate spheroidal P2VP particles, retaining the localized order previously induced by swelling. During subsequent pH cycles, swelling/deswelling was reversible. Finally, due to the electrostatically driven deformation of the microgel particles at the surface, the amount adsorbed was found to be smaller than that predicted for random sequential adsorption model for hard particles.

Addressing the temperature dependence of such dually electrostatically and thermally responsive physisorbed microgels, Wiedemair et al. investigated the thermoresponsive volume transition behavior of individual poly(N-isopropylacrylamide-*co*-acrylic acid) (PNIPAM-AAc) microgels physisorbed at amine-modified gold surfaces [34]. In doing so, AFM studies were performed by varying the force applied during imaging as a function of the geometry and material of the AFM probe. Low force impact magnetic excitation of the AFM probe during dynamic mode measurements was found to result in undisturbed deswelling behavior, enabling observation of the expected volume changes of the particles without significant tip-sample interaction. Elasticity measurements performed at single particles at temperatures revealed a 15-fold increase in the Young's modulus after passing the volume transition. Similarly, Wellert et al. investigated PNIPAM-AAc microgels in solution and adsorbed to poly(allylamine hydrochloride)-modified silicon [35]. The temperature-dependent deswelling was probed with AFM, while the inner structure of the adsorbed microgel particles was monitored by grazing incidence small angle neutron scattering, and the properties of the corresponding microgels in suspension probed by small-angle neutron scattering. In contrast to bulk measurements, the correlation length was found to remain constant for surface-bound microgels, indicating suppressed internal fluctuations. Furthermore, a temperature-dependent decrease in the specular peak intensity was observed, reflecting changes along the surface normal. Taken together, the results demonstrate an effective network stiffening of these anionic surface-bound microgels. Also, Burmistrova and Klitzing investigated temperature- and pH-sensitive PNIPAM-AAc microgels adsorbed on silicon wafers pre-coated with polyethyleneimine [36]. The amount of adsorbed microgel particles was controlled, e.g., by pH and microgel concentration. After surface deposition, the microgel particles were found to be highly compressed, but the temperature-responsive microgel swelling/deswelling remained reversible. The microgels were found to be strongly adsorbed at low ionic strength and intermediate pH. At higher ionic strength or in the acidic or basic regime, however, adsorption is much weaker, and microgel particles can be moved by the AFM tip or even desorb spontaneously. Furthermore, electrostatically induced microgel dimentional change occurred both laterally and normal to the surface. Similarly, Nerapusri et al. investigated close-packed monolayers of negatively charged PNIPAM and PNIPAM-AAc microgel particles onto poly(ethyleneimine)-modified silicon surfaces

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[37]. From measurements by spectroscopic ellipsometry, it was found that in the absence of ionizable comonomers, the temperature dependence of the adsorbed layer thickness mirrors that of the hydrodynamic diameter of microgels in solution. When ionizable carboxyl groups were introduced into the microgel particles, however, this correspondence was largely lost due pronounced flattening of the microgel particles on the oppositely charged surface.

While electrostatic swelling/deswelling behavior of charge microgels seems to be qualitatively retained after surface adsorption, despite considerable surface-induced structural changes, there have also been reports on the surfaces influencing the nature of the microgel transitions after surface deposition. For example, Burmistrova et al. investigated the swelling/deswelling behavior of PNIPAM and PNIPAM-AAc microgel particles in suspension and at the surface [38]. It was found that the two step volume phase transition, displayed by PNIPAM-AAc microgels in suspension, is lost after adsorption, and that the surface-bound microgels instead display a one step transition. Furthermore, the transition temperature was found to increase strongly with increasing charged co-monomer content, whereas mechanical studies showed the AAc-containing microgels to be softer than microgels in the absence of such co-monomers.

Investigating the effect of cross-linker content on the swelling behavior and elasticity of charged PNIPAM-AAc microgels, Burmistrova et al. studied swelling ratios of aqueous microgel suspensions by dynamic light scattering and compared the results to those obtained after adsorption at polycation-coated silicon wafers [39]. With increasing cross-linker content, the magnitude of the swelling/deswelling transition expectedly decreased. Furthermore, at high cross-linker content, the surface-bound microgels preserve their height/width ratio, while at low cross-linker content, height changes represent the dominating mode for swelling/deswelling. Mirroring this, Young's modulus measurements show that the microgels become stiffer with an increasing amount of cross-linker. On a related topic, Bachman et al. investigated PNIPAM microgels synthesized under 'crosslinker free' conditions. AFM nanoindentation of these ultralow cross-linked microgels indicate these to be considerably softer than traditionally cross-linked microgels, with a Young's modulus of ≈10 kPa [40]. Their high deformability was indicated also by a high degree of spreading on glass surfaces and by their ability to translocate through nanopores significantly smaller than the hydrodynamic diameter of the microgel particles. Due to this softness and deformability, ultralow cross-linked microgels constitute an interesting base material for biomedical applications, including biomaterials for drug delivery and regenerative medicine.

Finally, we note that swelling transitions in adsorbed microgels have been observed also for ampholyte microgels. For example, Schroeder et al. investigated such microgels based on poly(N-vinylcaprolactam) and PNIPAM in bulk solution and at glass surfaces [41]. Fourier transmission infrared spectroscopy and transmission electron microscopy confirmed the quantitative incorporation and random distribution of ionizable groups in the microgels, respectively. For balanced microgels (i.e., equal numbers of cationic and anionic groups), the pH-dependent size displays a symmetric minimum at zero net charge. The higher the fraction of cationic and anionic groups, the more pronounced the pH-dependent swelling/deswelling. Unexpectedly, however, the surface-bound microgels were found to be stiffer at pH 6.2 (i.e., at the isoelectric point), than above and below this pH.

As exemplified above, AFM has been extensively used to provide not only structural information of adsorbed microgels, but also for extracting information of the nanomechanical properties of such systems. As discussed by Dimitriadis et al., however, there are a number of challenges associated with such investigations, including the use of sharp cantilever tips, the use of the Hertz model for analyzing the AFM data, and uncertainties originating from difficulties in identifying the contact point in soft hydrogel systems [42]. Regarding the use of sharp cantilever tips, which typically induces local strains far exceeding the linear regime, these authors showed show that this problem can be resolved by using microsphere probes. Regarding the common use of the Hertz contact mechanics model, which leads to significant errors when applied to thin samples, a correction approach was presented. Moreover, error estimates were provided for the elastic modulus resulting from uncertainties related to the difficulty in establishing the contact point for soft materials. Demonstrating the validity of these approaches, theoretical and experimental results obtained for poly(vinyl alcohol) were compared to macroscopic measurements on poly(vinyl alcohol) gels.

3. Layer-by-layer structures containing microgels

A main strength of microgel-based surface coatings is their inherent simplicity, allowing interfacial deposition at widely different densities in a single step, either for pure microgel systems or their hybrids with inorganic nanomaterials, and with or without loading of cargo molecules (e.g., drugs). As such, microgel deposition is considerably more attractive from an industrial development perspective than more elaborate approaches, such as multistep layer-by-layer (LbL) formation. Nevertheless, microgel deposition has also been combined with LbL approaches in the design of more elaborate interfacial structures containing microgels. For example, Zavgorodnya and Serpe investigated temperature- and pH-sensitive PNIPAM-AAc microgels deposited on glass substrates coated with polyelectrolyte multilayers, in turn composed of cationic poly(allylamine hydrochloride) (PAH) and anionic poly (sodium 4-styrenesulfonate) (PSS) [43]. The microgel density and structure of the resultant films were investigated as a function of the number of PAH/PSS layers (i.e., the layer thickness), the charge of the outer layer of the polyelectrolyte multilayer film, and pH. From optical microscopy, AFM, and SEM, it was found that microgel charge played the most dominant role in determining the microgel surface coverage, although this is affected also by pH of the deposition solution, as well as the thickness and the outer layer charge of the PAH/PSS multilayer.

Similarly, Serpe and Lyon investigated the swelling behavior of PNIPAM-AAc microgel films as a function of pH using quartz crystal impedance (QCI) analysis and surface plasmon resonance (SPR) spectroscopy [44]. The microgels were assembled into thin films using a spin-coating LbL approach by alternatively exposing 2-mercaptoethylamine-functionalized Au substrates to polyanionic PNIPAM-AAc microgels and polycationic PAH. From QCI measurements, the deposited amount was found to depend linearly with the number of microgel layers. Furthermore, both QCI and SPR indicated the occurrence of a film transition, from being highly solvent-swollen under acidic conditions to being less swollen near neutral pH. This transition was found to be reversible, with the solvation/desolvation rates depending on the number of microgel layers in the film. In yet another study of such systems, Serpe et al. investigated the assembly of 4-acrylamidofluorescein-modified PNIPAM-AAc* microgel films [45]. Here, microgels were incorporated into films by alternatively exposing glass slides functionalized with 3-aminopropyltrimethoxysilane to anionic PNIPAM-AAc* microgels and cationic PAH. As expected from the solution behavior of the microgels, the films exhibited deswelling and enhanced thermoresponsivity below pKa of the microgel acrylic acid groups.

Clarke and Lyon investigated the possibilities to fine-tune thermoresponsiveness of microgel-based LbL structures [46]. In doing so, microgels were prepared by copolymerization of N-isopropylacrylamide and N-isopropylmethacrylamide in varying ratios, followed by LbL deposition of these microgels and cationic PDADMAC. After deposition, these microgels were found to retain the temperature response displayed in suspension, as demonstrated by temperature-dependent light scattering (Fig. 5). Furthermore, multilayers composed of more than one type of microgel were demonstrated to display multiple temperature responses similar to those observed for the individual building blocks, permitting fine-tuning of the temperature responsive interface. From experiments with mixed composition films and

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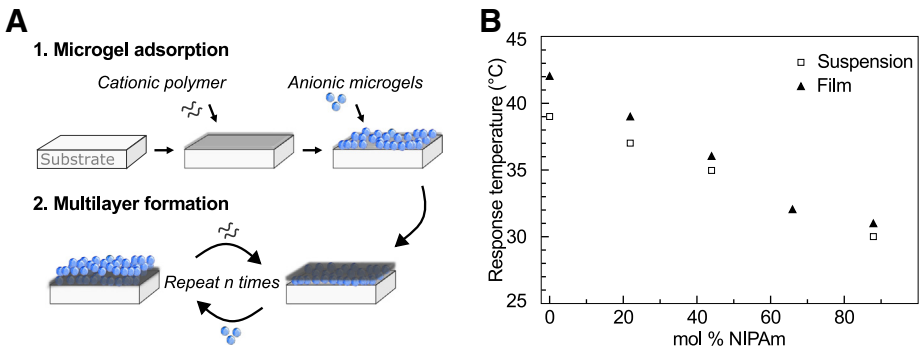

Fig. 5. Microgel films can be constructed by LbL approaches, in which adsorption of (frequently charged) microgels is alternated by the adsorption of (frequently oppositely charged) polymers. By charge over-compensation, the surface obtains a net charge after each adsorption step, primarily determined by the composition of the outer layer, in turn allowing another layer to de deposited. Through this, multiple layers can be deposited (a), with the microgel surface load essentially increasing linearly with the number of deposition cycles (b). The responsiveness of such microgel-based multilayers maintains the responsiveness as the individual microgel particles, as demonstrated for 6-layer PNIPAM/PNIPMAM microgel/PDADMAC films. As seen, the phase transition temperature of such multilayer films displays the same dependence on the NIPAM content as the individual microgel particles. (Re-drawn from [46].)

multiple assembly processes, it was also shown that the location of the microgels within the film does not affect their temperature response, indicating that microgels within the LbL film behave independently of neighboring microgels with respect to their thermally induced deswelling.

Although these studies have shown that microgels retain most of their responsiveness also when incorporated into LbL structures, there have also been reports that the multi-component nature of such assemblies may affect of the microgel behavior. For example, Sorrell and Lyon employed QCM, SPR, and AFM to investigate films formed by PNIPAM-AAc microgels and PAH, using spin-coating LbL assembly [47]. It was found that the interaction between the negatively charged microgels and positively charged PAH significantly influences the pH responsivity of the film, as seen both from their optical and mechanical behavior. Furthermore, slight changes to the film architecture and ambient pH was observed in both QCM and SPR responses, suggesting that the pH-dependent swelling depends on both particle swelling and polyelectrolyte de-complexation.

4. Microgel layers obtained from spin-coating and directed self-assembly

Due to the low surface coverages typically obtained by simple adsorption (jamming ≈0.5–0.6 in random sequential adsorption), directed self-assembly and forced deposition have attracted interest as ways to deposit microgels at higher surface coverages, and through faster and more reproducible processes. Reaching higher surface densities, all the way up to two-dimensional (2D) or three-dimensional (3D) colloidal crystals, is of importance in particular for optical and sensing applications, for which the soft-sphere character and the stimuli-responsive behavior of microgels provide not only a flexible design platform, but also potent triggers for assembly and disassembly. Investigating forced deposition as a way to reach such higher particle densities, Schmidt et al. reported on thermoresponsive coatings fabricated from spin-coating of PNIPAM-AAc microgels on silicon wafers [48]. At pH 2, the microgel particles are weakly negatively charged due to the polymerization initiator used, forming surface layers with dense packing and strong flattening, an effect particularly pronounced for microgels containing less cross-linker. Coatings consisting of these microgels display reversible thermoresponsive swelling/deswelling around the lower critical solution temperature of PNIPAM. While the temperature-induced transition was found to be sharp for microgels with lower amount of cross-linker, it was observed to become more gradual with increasing cross-linker content. Similarly, Singh et al. reported on spin-coating of PNIPAM microgels as a way for functionalization of poly(ethylene terephthalate) (PET) with a dense microgel layer, subsequently stabilized by covalent attachment to the PET surface via a photoaffinity technique [49].

For optical reasons, ordered 2D and 3D microgel assemblies displaying colloidal crystal ordering are of interest, e.g., in biosensor applications. Testing various particle deposition methods for generating such structures, Schmidt et al. investigated the deposition of PNIPAM-AAc microgel particles on polyelectrolyte-modified silicon wafers, as well as effects of pH, substrate precoating, and preparation technique [50]. Based on AFM and ellipsometry, it was found that both pH and the surface deposition technique used significantly influence the adsorption density, while the substrate surface charge is of less importance, suggesting that particle-particle interactions, rather than particle-surface interactions, determine the adsorption density of these microgels, in analogy to numerous other colloidal particles and particle-like macromolecules (e.g., globular proteins). In addition, higher surface roughness was found to lead to particle aggregation, which was inferred as being due to “wrapping” by the dangling ends and loops on rough surfaces, leading to an effectively higher number of binding sites. Furthermore, washing after preparation was found to affect the particle distribution, notably resulting in more ordered patterns.

Investigating the effect of particle concentration on the formation of microgel crystals, Debord and Lyon employed confocal and differential interference contrast microscopies to directly observe the packing in entropic crystals formed by PNIPAM and PNIPAM-AAc microgels [51]. Close-packed ordering was observed when the microgel particles were concentrated by centrifugation. After deposition, the particle-particle spacing was found to be smaller than the hydrodynamic diameter as determined by photon correlation spectroscopy, demonstrating compression of the soft microgel particles. Interestingly, excellent crystallization was observed even after extensive dilution of the acrylic acid-containing particles, despite a doubling of the center-to-center particle spacing. These results are not explainable by simple hard sphere packing models, which would predict crystal melting upon dilution, and were instead interpreted in terms of particle compressibility (for higher concentration samples) as well as an attractive pair- or multibody potential (for low-concentration samples).

Similarly, Tsuji and Kawaguchi investigated how PNIPAM microgels and PNIPAM-coated polystyrene-based particles self-assemble into a 2D superlattice when their dilute dispersions were dried on poly(styrene), glass, silicon, and Au substrates [52]. Again, the capillary force acting on the particles during drying was found to result in ordered array formation, where PNIPAM provided steric stabilization. Tsuji and Kawaguchi also investigated ordered 2D structures composed of PNIPAM microgel particles prepared by deposition of a droplet of the microgel dispersion on glass or polystyrene substrates. AFM observation of the resulting

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films revealed these to consist of a monolayer particle array, producing iridescent colors due to optical diffraction in the ordered structure [53].

In yet another study on this topic, Horecha et al. investigated on-surface self-assembly of microgels prepared via precipitation polymerization of N-isopropylacrylamide with methylenebisacrylamide, acrylic acid, or acrylamide as co-monomers into periodic loosely packed 2D arrays [54]. The main factor controlling microgel separation and driving array formation was found to be the presence of large low-density shells around the highly cross-linked microgel cores. The arrays thus formed were subsequently used as masks for silane modification, whereas sputtering of gold onto the periodic microgel arrays was demonstrated to be an efficient way of preparing perforated gold films with array holes.

A different approach of forced assembly of microgels at surfaces is that of controlled wrinkling of a soft substrate with a hard top layer [55]. The basis for this is that a system comprised of a thin rigid layer on top of an elastic basis buckles as the compressive force exceeds a critical value. The wavelength and amplitude of the wrinkles formed depend on the mechanical properties of the top layer and the basis, as well as on the applied stress. Employing this approach for microgels, Hitl et al. investigated guided large scale self-assembly of microgels consisting of N-vinylcaprolactam, N-isopropylacrylamide, and acetoacetoxyethyl methacrylate, using nanostructured substrates fabricated via controlled wrinkling [55]. Depending on microgel, the structure arrangement was found to influence both particle shape and the overall microgel pattern formation. Thus, both straight lines composed of single particles and zig-zag structures were observed. In an additional processing step, these nanostructured substrates were used as stamps to transfer the formed microgel patterns onto a planar support (Fig. 6).

A crucial step in template-assisted nanoparticle assembly is the adjustment of characteristic template dimensions (e.g. trench width, depth, and separation) to the particle size, surface charge, and concentration. This necessitates extensive screening of possible combinations, a time-consuming task when using photolithography and laser writing techniques. In an attempt to address this, Hiltl et al. developed nanostructured gradient wrinkle surfaces based on oxidized top layers on elastic poly(dimethylsiloxane) substrates to establish a one-step screening process towards optimal assembly of soft and hard colloidal particles (microgel and silica particles) [56]. Through a combinatorial approach, these authors optimized particle assembly with respect to the ratio of particle diameter to wrinkle wavelength and packing density, as well as to differences between soft and hard particles. Using this approach, together with partial shielding of the substrate during plasma oxidation, allowed the formation of 2D gradients with amplitudes of 7–230 nm and wavelengths of 250–900 nm.

Pushing the approach towards hybrid nanomaterials, Müller et al. investigated wrinkle-assisted assembly of core–shell particles with hard (silica or silver) cores and soft PNIPAM shells [57]. Anisotropic alignment was observed on two length scales, one macroscopic, guided through the wrinkle structure, and one local due to deformation of the polymer shell. Radial distribution functions obtained from SEM demonstrated the impact of confinement on nearest neighbour distances and symmetry. The observed ordering was compared to Monte Carlo simulations for hard-core/soft-shell particles, showing that the observed symmetries are determined by the soft interaction potential, differing qualitatively from the hard-sphere situation. For silver-PNIPAM particles, UV-VIS absorbance measurements demonstrated optical anisotropy of the generated structures due to plasmon coupling. Furthermore, the high degree of order of the assembled structures on macroscopic areas was demonstrated by laser diffraction. Similarly, Jaber et al., demonstrated self-assembly of nanocrystal monolayers from core-shell gold nanocrystals with a thermoresponsive polymer shell by either spin-coating or convective assembly, interparticle spacings being widely tunable by the polymer shell thickness [58]. Using such an approach, 2D arrays of Au-PNIPAM core-shell nanocrystals fabricated using convective deposition and spin-coating were investigated. Subsequent annealing at 700 °C was used to remove the polymer shell, resulting in a monolayer of well-separated gold nanoparticles, the plasmon modes of which were analyzed by spectroscopic ellipsometry.

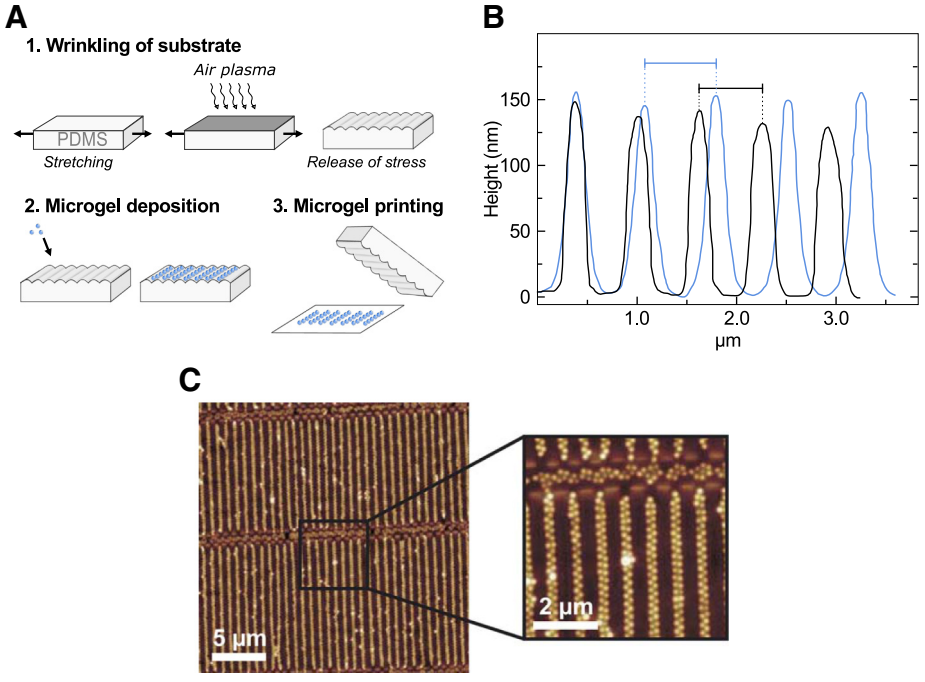

Fig. 6. (a) Schematic illustration of the principles of wrinkling-assisted microgel assembly at surfaces. Stretched poly(dimethylsiloxane) was plasma treated to generate a stiff silica overlayer, causing the surface to wrinkle on releasing the stress. If a microgel dispersion is spin-coated or otherwise deposited on such surfaces, microgel particles will deposit into the grooves of the substrate, which in turn can be controlled by film thickness, strength, degree of stretching, and various other parameters. (b) Through wrinkling assisted assembly, ordered microgel structures can be deposited at surface, illustrated by N-vinylcaprolactam/acetoacetoxyethyl methacrylate and N-isopropylacrylamide/N-vinylcaprolactam microgels deposited on silicon wafers. (Re-drawn from [55].) (c) Representative AFM images of N-vinylcaprolactam/acetoacetoxyethyl methacrylate microgels deposited on wrinkles roughly 780 nm apart. Height scale 150 nm.
(Reproduced from [55] with permission from the Royal Society of Chemistry.)

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5. Macroscopic microgel-containing films

As discussed above, formation of thick, microgel-based, surface coatings has attracted some interest in literature. Apart from the more elaborate LbL assemblies, research on such systems has included also work on films, in which microgels are either polymerized after deposition, or *co*-polymerized in a polymer matrix. For example, McGillicuddy et al. designed "plum pudding" gels, prepared from thermoresponsive microgel particles formed by N-isopropylacrylamide (NiPAAm) and N-tert-butylacrylamide (NtBAAm), which were then re-suspended into ethanol solutions containing also NiPAAm/NtBAAm, and cast on a surface [59]. The resulting surface coating was evaluated as a drug-containing coating of stainless steel stent wires for the treatment of restenosis. Furthermore, Nayak et al. investigated the deswelling behavior of PNIPAM-based microcomposite hydrogel films, formed by precipitation-polymerized PNIPAM microgel particles, in turn polymerized into a PNIPAM gel matrix immobilized on a glass slide [60]. In conventional (not microstructured) films, turbidity was found to increase and plateau as the temperature was increased above the lower critical solution temperature (LCST) of PNIPAM. In contrast, microgel composite films displayed an increase in turbidity until the LCST, followed by a rapid turbidity decrease above the LCST. Furthermore, the rate at which light scattering decreases was found to depend on the microgel concentration in the film. These results indicate that the microgel particles collapse independently of the surrounding polymer network. Since the cross-link density of the microgels is much lower than that of the matrix, and since PNIPAM microgels with lower cross-linking densities have a lower phase transition temperature, the microgel particles are expected to deswell first on increasing temperature.

Microgel-based composite films have been investigated also for polymeric but non-cross-linked materials. For example, Chen et al. investigated the morphology and light absorption properties of spin-coated composite films prepared from mixed dispersions of polystyrene microgels and poly(3-hexylthiophene) (P3HT) [61]. The films were observed to contain flattened microgel particles with an aspect ratio of ≈ 10. Furthermore, microgel islands containing hexagonally close packed particles were found for both the microgel and microgel/P3HT composite films. In addition, the morphology of the composite film was demonstrated to depend on the microgel and P3HT concentration, and a morphology phase diagram constructed. The P3HT phase acted as electrically conducting cement and increased the robustness of the films to solvent washing. The P3HT also rendered the composite films photoactive, with an absorbance increasing linearly with both microgel and P3HT concentration.

Finally, we note the possibility of forming composite films by microgels in the presence of inorganic nano- and microparticles. For example, Pinprayoon et al. investigated polymer films prepared from cross-linked poly(butadiene/methacrylic acid) (poly(Bd)/poly(MAA)) particles, also containing ZnO particles as a source of Zn^{2+} [62]. FTIR data revealed that the Zn^{2+} ions formed ionic cross-links with the polymer carboxylate groups. Mechanical property studies and stress-strain measurements revealed the existence of two main phases in the systems, one poly(Bd)-rich and one poly(MAA)-rich. A third phase was also found, ascribed as an interphase between the particle core and shell. Furthermore, SAXS showed that the films were composed of distinct poly(Bd/MAA) particles, implying that only partial coalescence occurred across the particle interfaces during formation of these particulate ionomer films. SAXS also showed scattering from $Zn^{2+}/RCOO^{-}$ ionic aggregates, demonstrating the key role of Zn^{2+} in the formation of these films.

6. Biomedical applications of surface-bound microgels

6.1. Biosensors

Since microgels can be designed to undergo dramatic swelling/deswelling transitions in response to a wide range of triggers, such as temperature, pH, ionic strength, redox conditions, presence of specific ions, metabolites, and antigens/antibodies, but also to conformational changes, specific biological cascades and other triggers, surface-bound microgels offer considerable opportunities in biosensor applications. Particular focus in such applications has been placed on etalons, in which microgels are introduced as spacers between two metal surfaces [8]. As the microgels undergo swelling/deswelling in response to some triggering parameter, the spacing between the two metal substrates change, allowing detection, e.g., by optical methods. Demonstrating the considerable signal shifts displayed by such devices, Sorell et al. reported on the possibility to introduce color tunability over 300 nm in response to a 15 °C temperature change by assembling thermoresponsive PNIPAM-AAc microgels on an Au-coated glass substrate, followed by addition of another Cr/Au layer as an overlayer [63]. Much like a Fabry-Pérot etalon or interferometer, the optical effects observed are due to temperature-induced changes in microgel swelling, which in turn affects the spacing between the two Au layers. In addition, the films are pH responsive, such that the temperature-induced color change was suppressed at high pH, i.e., at conditions where the carboxyl groups are fully dissociated and the temperature-responsiveness of these microgels suppressed.

Extending the use of etalons for detection of bioanalytes, Islam and Serpe designed an etalon assay based on PNIPAM-AAc microgels. Demonstrating the assay for the determination of streptavidin, complementary avidin was first coupled to biotin-conjugated PAH. After equilibration of the streptavidin-containing sample with (an excess of) this conjugate, the resulting streptavidin-biotin-PAH complexes were removed by subsequent complexation with biotin-conjugated magnetic nanoparticles and magnetic separation. On contacting the remaining solution with the PNIPAM-AAC etalon, PAH-biotin resulted in electrostatically triggered microgel deswelling. The change in interference caused by this could then be related to the PAH-biotin concentration, in turn allowing the streptavidin concentration of the original sample to be extracted (Fig. 7) [64].

Analogously, Zhang et al. investigated Au surface deposition of PNIPAM microgels containing triphenylmethane leucohydroxide [65]. The optical properties of the resultant etalons were investigated, as was their response to ultraviolet and visible irradiation, solution pH changes, and the presence of nerve agent mimic. Pronounced changes in optical properties were found for all these stimuli. Furthermore, by applying independent drops of solutions to the device surface, Hu and Serpe were able to demonstrate that spatially isolated regions of a single PNIPAM-AAc microgel-based etalon may display independent responses to solution pH and temperature [8]. In addition, PNIPAM-AAc microgel-based etalons are able to display responses to different salts [66] Illustrating this for K^{+}, Na^{+} and NH_4^{+}, Hu and Serpe characterized the hysteresis on cycling solution pH, and demonstrated this to depend dramatically on salt composition [67]. In addition, the etalon signal obtained depends on salt concentration, as demonstrated, e.g., for NaCl. Microgel-based etalons have also been demonstrated to provide opportunities in various other contexts, e.g., for the detection of polymer molecular weight [68]. These and other examples illustrate the versatility of microgel-based etalons, with potential applicability for, e.g., remote sensing/actuation and color-tunable optics, as nicely discussed in larger detail by Gao et al. [69] and Islam et al. [66].

Although the largest focus on the use of microgels as biosensor components has been placed on etalons and related hybrid constructs, surface-bound microgels can be used also on their own in this context, without metal films for interference generation. For example, Kim et al. investigated bioresponsive microgels ("hydrogel microlenses") as a tool for label-free protein detection, for which stimuli-responsive PNIPAM-AAc microgels were synthesized via free-radical precipitation polymerization and subsequently functionalized with biotin [70–72]. Arrays of hydrogel microlenses were then prepared by assembly onto a silane-modified glass substrate. Multivalent binding of both avidin and polyclonal anti-biotin effectively induced cross-links in the

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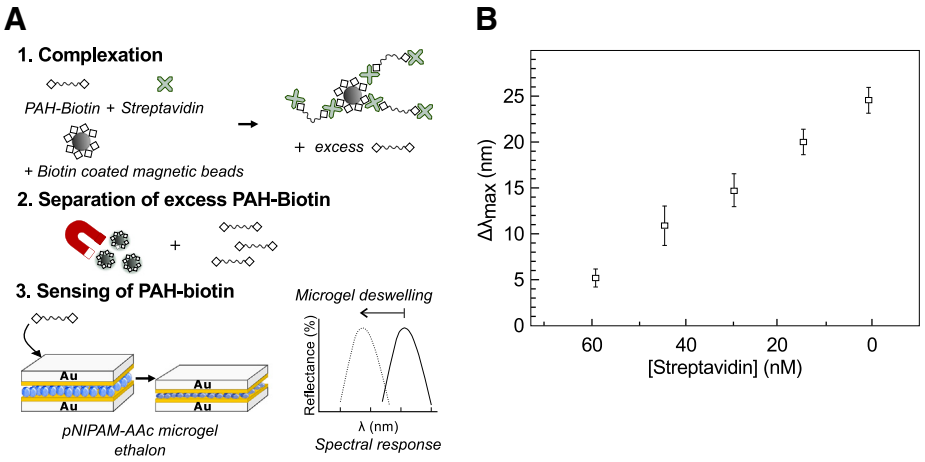

Fig. 7. Microgel-based etalons allow sensitive label-free analysis of protein analytes. (a) Schematic illustration of the principles for determination of streptavidin. For this, complementary avidin was first coupled to biotin-conjugated PAH. After equilibration of the streptavidin-containing sample with (an excess of) this conjugate, the resulting streptavidin-biotin-PAH complexes were removed by subsequent complexation with biotin-conjugated magnetic nanoparticles and magnetic separation. On contacting the remaining solution with the PNIPAM-AAC etalon, PAH-biotin resulted in electrostatically triggered microgel deswelling. The change in interference caused by this could then be related to the PAH-biotin concentration, in turn allowing the streptavidin concentration of the original sample to be extracted. (b) Using this assay allowed sensitive label-free detection of streptavidin, using principles, which could straightforwardly be extended to any biological affinity pair (e.g., antigen-antibody pairs or complementary nucleotide stretches).
(Re-drawn from [64].)

microgels, which could be monitored by changes in their optical properties. Due to the potent antifouling properties of the microgels, protein binding is highly specific for the target protein, with no detectable interference from non-specific protein binding. In analogy with macroscopic solid phase diagnostic methods (e.g., ELISA), binding-induced antibody-antigen cross-links can be disrupted by the introduction of free antigen to the surrounding medium for competition assays. As the antibody is covalently tethered to the microlens, washing with antigen-free media results in re-assembly of the tethered antibody/antigen pairs, thereby providing for reversible biosensing. Taken together, these findings suggest that the microlens method have potential for label-free detection of proteins or small molecules via displacement of tethered protein-ligand pairs.

Also other approaches for using surface-bound microgels in biosensor applications can be noted, including electrochemistry-based ones. For example, Sigolaeva et al. investigated enzyme-loaded microgel films adsorbed onto conductive graphite or gold substrates [73]. For this, a pH-sensitive microgel was synthesized by precipitation copolymerization of N-isopropylacrylamide (NIPAM) and dimethylaminopropylmethacrylamide (DMAPMA). Enhanced deposition of the resulting poly(NIPAM-*co*-DMAPMA) microgel particles was found at temperatures above the volume phase transition temperature (VPTT). In addition, loading of tyrosinase to such films was examined at different adsorption regimes, and enhanced enzyme loading obtained when the microgel film was brought into a collapsed state before equilibration with the enzyme at T < VPTT. By temperature-induced stimulation of both (i) poly(NIPAM-*co*-DMAPMA) microgel adsorption at T > VPTT and (ii) tyrosinase loading at T < VPTT, a > 3.5-fold increase in biosensor sensitivity for phenol was obtained (Fig. 8).

6.2. Drug delivery

For microgels in suspension, it has been demonstrated that loading of drugs and other cargo molecules into microgels, as well as their subsequent release, depends on numerous factors, including, e.g., the solubility and the size of the guest molecule in relation the mesh size of the microgel network. For example, loading of peptides into microgels depends on peptide length [74,75], hydrophobicity (distribution) [76], charge (distribution) [77], nature of the charged group [78], and secondary structure [79]. In addition, microgel network properties, such as net charge and mesh size, affect peptide/protein loading and release, as do solution conditions such as pH and ionic strength [74,77]. Furthermore, several of these properties have been demonstrated to influence protection of microgel-loaded peptides and proteins against proteolytic degradation [80], and microgel degradation in turn to depend on peptide/protein loading [81]. Also for other drug types, loading into, and release from, microgels depend on these properties in analogous ways [4–6].

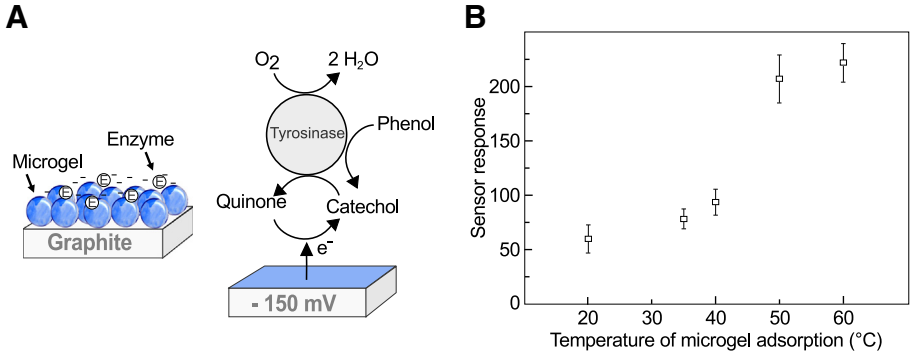

Fig. 8. (a) Schematic illustration of surface-bound poly(NIPAM-*co*-DMAPMA) microgels, their loading with tyrosinase, and the subsequent use of such functionalized electrode surfaces for electrochemical determination of phenol. (b) The enzymatic electrochemical activity of tyrosinase in such films depends on temperature-controlled microgel swelling, and enzyme loading could be significantly enhanced for improved sensitivity through temperature-cycling of such systems (c). Phenol concentration in (b) and (c) was 1 μM.
(Re-drawn from [73].)

In contrast to microgels in suspension, less is known about loading and release into surface-bound microgels (Fig. 9). Addressing this in the context of peptide delivery, Nyström et al. investigated the effects of electrostatics and peptide size on peptide interactions with surface-bound microgels, using ellipsometry, confocal microscopy, and AFM [82]. Results show that binding of cationic poly-L-lysine (pLys) to anionic, covalently immobilized, poly(ethyl acrylate-co-methacrylic acid) microgels increased with increasing peptide net charge and microgel charge density. Furthermore, peptide release was facilitated by decreasing either microgel or peptide charge density (Fig. 9a). Analogously, increasing ionic strength facilitated peptide release for short peptides. As a result of peptide binding, the surface-bound microgels displayed pronounced deswelling and increased mechanical rigidity, the latter quantified by quantitative nanomechanical mapping. While short pLys peptides were found to penetrate the entire microgel network and to result in almost complete charge neutralization, larger peptides were partially excluded from the microgel network, forming an outer peptide layer on the microgels. As a result of this difference, microgel flattening was more influenced by the lower Mw peptide than the higher. Peptide-induced deswelling was found to be lower for higher Mw pLys, the latter effect not observed for the corresponding microgels in the dispersed state. While the effects of electrostatics on peptide loading and release were similar to those observed for dispersed microgels, there were thus considerable effects of the underlying surface on peptide-induced microgel deswelling.

Similarly addressing loading and release of low-Mw organic cargo compounds, FitzGerald et al. investigated the pH-responsive behavior for a series of lightly cross-linked, sterically stabilized poly(tertiary amine methacrylate)-based latexes adsorbed onto mica and silica by in situ tapping mode AFM. The adsorbed layer structure was primarily determined by the glass transition temperature, Tg, of the polymer particles [83]. Thus, low Tg poly[2-(diethylamino)ethyl methacrylate]-based particles were found to form uniform films, whereas higher Tg poly[2-(diisopropylamino)ethyl methacrylate] retained their original particulate character. Both polymer particles displayed cationic microgel character and swelled appreciably at low pH, even those that had coalesced to form films. Fluorescence spectroscopy was used to study the capture of a model hydrophobic probe, pyrene, by the adsorbed microgels, followed by its subsequent release by lowering the solution pH. Through this, the repeated capture and release of pyrene through several pH cycles could be demonstrated.

Of more direct relevance for drug delivery, Wang et al. investigated loading and release of the anti-inflammatory drug ibuprofen to/from surgical sutures. For this, the sutures were coated with multilayer films containing microgels of chemically cross-linked poly(allylamine hydrochloride) and dextran (PAH-D) through layer-by-layer deposition. Ibuprofen incorporated in such coated sutures was found to display sustained release over ≈100 h in physiological salt concentration [84]. Ibuprophen release from microgel-based LbL films was investigated also by Wang et al. [85] and Zhang et al. [86] for other substrates. Furthermore, Serpe et al. investigated thermally regulated uptake and release of the chemotherapeutic drug doxorubicin from microgel thin films [87]. For this, spin coating-based LbL assembly was used to prepare films composed of PNIPAM-AAc microgels by alternatively exposing a 3-aminopropyltrimethoxysilane (APTMS)-functionalized glass substrate to polyanionic PNIPAM-AAc microgels and polycationic PAH. Using this method, up to 30 microgel layer films were generated with uniform layer buildup, as confirmed by QCM. The films were subsequently loaded with doxorubicin by temperature cycling in an aqueous doxorubicin solution. It was found that doxorubicin loading was reduced at low pH, where the PNIPAM-AAc microgel and the basic doxorubicin are both weakly positively charged. In addition, doxorubicin release was found to increase on cycling temperature between 20 and 50 °C.

Investigating similar systems for delivery of peptide therapeutics, Nolan et al. investigated thermally triggered insulin release from

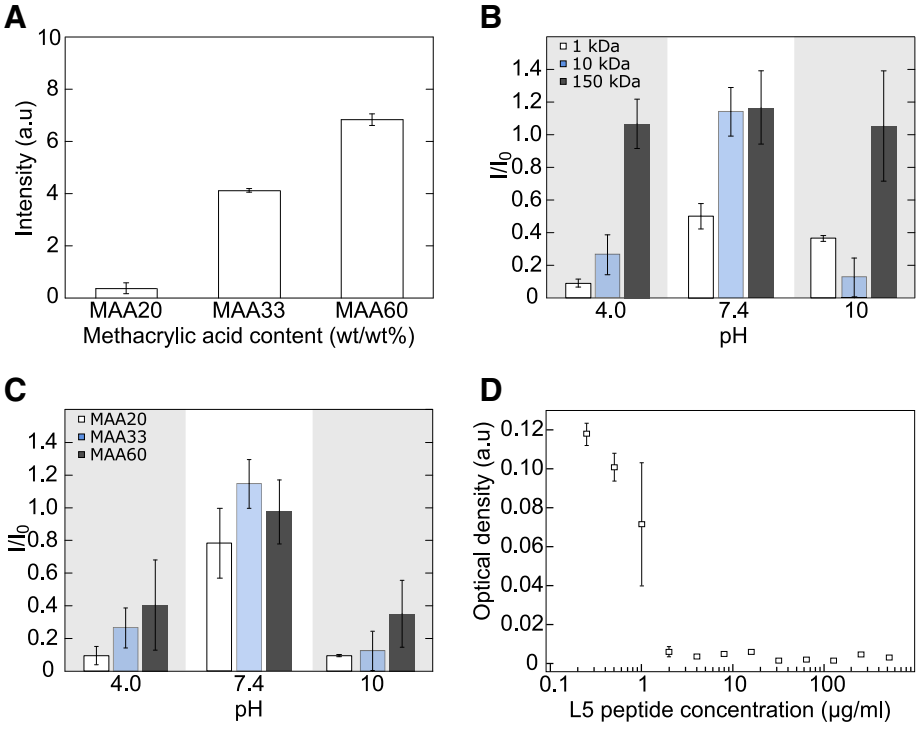

Fig. 9. Loading and release of antimicrobial peptides into/from surface-bound microgels depend on microgel charge, ambient conditions, and microgel charge density, and can be used to provide release-induced antimicrobial effects. (a) Loading of poly(lysine) to surface-bound poly(ethyl acrylate-*co*-methacrylic acid) microgels (33 mol% methacrylic acid) increases with peptide net charge and microgel charge density, while peptide release is promoted by decreasing peptide and microgel charge density (b,c). In the latter experiments, peptides were loaded in 10 mM Tris, pH 7.4, releasing the peptide at the same ionic strength and the indicated pH. *(Redrawn from [82].)* (d) Antimicrobial effect of surface-bound and L5-loaded poly(ethylene glycol)-co-acrylic acid microgels against *S. epidermidis*.
(Re-drawn from [96].)

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PNIPAM-AAc microgel films prepared by LbL assembly [88,89]. Interestingly, the films were found to deswell faster than the insulin release rate, suggesting that the latter is not solely limited to the volume exclusion caused by the collapse of the network, but also regulated by solubility or partitioning effects. Despite this, film thermosresponsivity plays an indirect role for drug release, in that subjection to many thermal cycles enables the embedded peptide to solubilize and subsequently partition through an increasing number of film layers. Through pulsatile and extended release studies, it was demonstrated that these films are able to release insulin bursts over many cycles, the magnitude of which can be controlled by the film thickness. Furthermore, the insulin-impregnated films were demonstrated to be extremely stable, able to release constant pulses of peptide for >1 month at a time (Fig. 10).

In one of few studies so far on the performance of surface-bound microgels as drug delivery systems, McGillicuddy et al. evaluated "plum pudding" gels as potential drug-eluting stent coatings, with particular focus on the controlled delivery of fluvastatin from intravascular stent surfaces as a potential therapeutic approach for prevention of in-stent restenosis [59]. In doing so, gels were prepared from fluvastatin-loaded thermoresponsive microgel particles formed by N-isopropylacrylamide (NiPAAm) and more hydrophobic N-tert-butylacrylamide (NtBAAm), which were subsequently re-suspended into ethanol solutions containing also NiPAAm/NtBAAm, and then cast on a surface. Fluvastatin release from such copolymer films expectedly decreased with increasing film hydrophobicity, and could be sustained over an extended period for optimized systems. Importantly, the eluted drug retained its bioactivity, assessed as selective inhibition of human coronary artery smooth muscle cell proliferation. The use of such materials was also demonstrated for stainless steel stent wires, showing that drug elution into static and perfused flow environments followed similar elution profiles as for the model substrates.

6.3. Functional biomaterials

Addressing the potential use of surface-bound microgels as coatings for biomaterials, Gan and Lyon investigated thermoresponsive PNIPAM nanoparticles grafted with poly(ethylene glycol) (PEG), obtained through free-radical precipitation polymerization, using PEG monomethyl ether monomethacrylate (Mw = 1 kDa) as a co- "monomer" [90]. The challenges connected to PEGylating microgels, such as broadening of the particle size distributions, broadening of the volume phase transition of the microgels, and a shift of the latter to higher temperatures, were minimized by localizing the PEG chains to the particle periphery using a two-stage precipitation polymerization method. In analogy to numerous previous studies on PEGylation of both macroscopic surfaces and particles/macromolecules [91,92], protein adsorption to the microgel was found to be suppressed as a result of PEG incorporation into the particles, especially when the PEG chains are located in the microgel shell. Both protein adsorption measurements and ^{1}H NMR studies suggested that the PEG side chains stretch outward from the particle surface, also as the particles collapse at temperature above the phase transition temperature. Interestingly, analogous effects were observed for particles where the PEG chains are localized in the particle core, suggesting that the PEG grafts can penetrate the PNIPAM shell when it is in its phase-separated state.

Analogously, Nolan et al. investigated PNIPAM microgel particles cross-linked with various concentrations of PEG diacrylates of different chain length, monitoring the phase transition and protein adsorption [93]. Based on light scattering, an increase of the temperature and width of the phase transition was found with an increasing concentration of PEG cross-linker incorporated into the microgels. Qualitative differences in particle density using isopycnic centrifugation showed that denser microgel networks are obtained at higher PEG concentrations. Based on NMR studies in the deswollen state, it was also inferred that the longer PEG cross-links protrude from the dense globular network, resulting in a decrease in non-specific protein adsorption with increasing PEG length and content. In analogy, surface-bound microgels containing longer PEG chain lengths exhibited enhanced non-fouling and were resistant to cell adhesion in serum-containing media (Fig. 11). Analogous suppression of protein adsorption and cell adhesion was observed by Scott et al. for microgel assemblies formed by octavinylsulfone-modified PEG and bovine serum albumin [94]. Considering this, as well as the simplicity of (geometry-independent) surface modification, PEGylated microgels are of potential interest for applications where non-fouling coatings are required.

In an attempt to further improve the performance of microgel-based coatings through increasing microgel packing density at the surface, South et al. investigated the use of centrifugation-based assembly of microgel films [95]. In doing so, films assembled from microgel particles were obtained from either centrifugation ("active") deposition or dip coating adsorption ("passive"). It was found that microgels actively deposited onto a surface have smaller footprints and are more closely packed, in analogy to reduced surface-induced spreading of globular proteins under high flux deposition. Taking advantage of this, the use of active deposition for the fabrication of polyelectrolyte multilayers containing anionic microgels and a cationic linear polymer was demonstrated. Such microgel multilayers displayed effective blocking of the underlying substrate in relation to macrophage adhesion, of interest, e.g., for modulating the inflammatory response to implanted biomaterials.

Furthermore, Wang et al. explored the use of self-assembled microgels to inhibit the bacterial colonization of synthetic surfaces [96]. In doing so, two antimicrobial mechanisms were investigated, i.e., i) modulation of surface cell adhesiveness and ii) local storage/

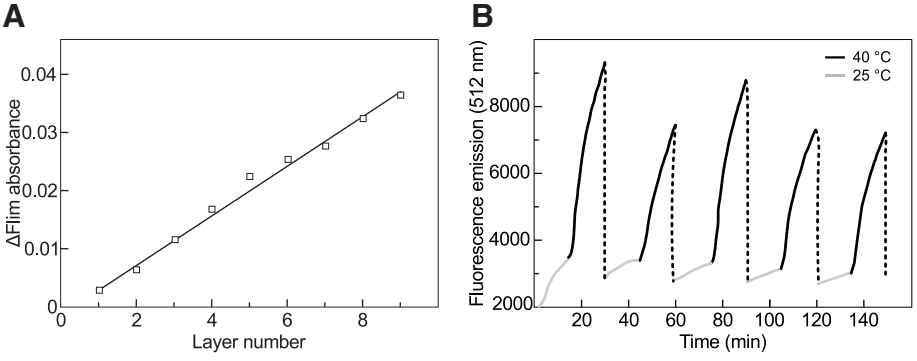

Fig. 10. (a) Loading of FITC-insulin into multilayers formed by repeated spin-coating of PNIPAM-AAc microgels (10 mol% AAc) onto PAH-modified glass substrates increases linearly with the number of layers deposited. (b) Pulsatile release of FITC-insulin from a nine-layer microgel multilayer into 20 mM PBS buffer, pH 7.4. Black and gray lines represent T = 25 °C and T = 40 °C, respectively, while dotted lines indicate full buffer replacement.
(Re-drawn from [88].)

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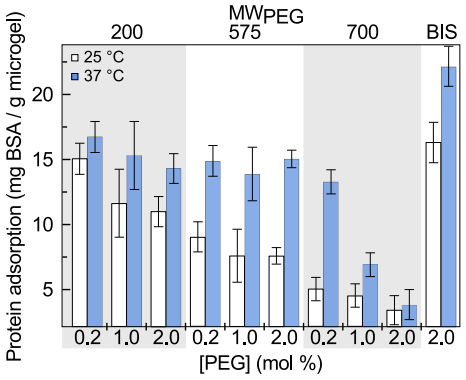

Fig. 11. Adsorption of bovine serum albumin to PNIPAM-based microgels from 20 mM PBS buffer, pH 7.4, as a function of PEG content and molecular weight. With increasing PEG molecular weight and content, protein adsorption is reduced at 25 °C, where the microgels are highly swollen. In contrast, protein adsorption remains high for deswollen microgels at 37 °C, until the PEG chains are sufficiently long to be able to protrude all the way to the microgel exterior.
(Re-drawn from [93].)

release of an antimicrobial compounds. For this, PEG and PEG-co-acrylic acid (PEG-AAc) microgels were synthesized by suspension photopolymerization, and the resulting microgels deposited onto poly(L-lysine)-primed silicon to form sub-monolayer surface coatings. After deposition, the microgels were loaded with a cationic antimicrobial peptide (L5), peptide loading being significantly higher in PEG-AA microgels than in pure PEG microgels for electrostatic reasons. It was found that coating the silicon substrate by peptide-free PEG-AAc microgels considerably reduced the short-term *S. epidermidis* surface colonization, the degree of inhibition increasing with decreasing average spacing between the surface-bound microgels. Post-deposition L5 peptide loading into microgels further reduced *S. epidermidis* colonization to the very small level of colonization observed for macroscopic PEG gel controls (Fig. 9d).

Addressing the granular nature of microgel films under sub-confluence coverage, Wang and Libera investigated surface deposition of microgels formed by suspension polymerization of AAc and PEG to polylysine-modified silicon surfaces [97]. It was found that the surface-bound PEG-AA microgels efficiently resist fibronectin adsorption. In contrast, poly(lysine) exposed between the adhered microgel particles (sub-confluent coating) readily adsorbs this protein, thereby producing a disordered array of submicron-sized non-adhesive regions on an otherwise cell adhesive surface. Compared to continuously adhesive surfaces, the microgel-coated surfaces were found to result in short-term cell spreading and cell proliferation, while the differentiation behavior was unaffected. Using SEM, it was shown that osteoblasts grow over the microgels, adhering to the poly(lysine)-exposed areas of the surface, while time-resolved optical microscopy demonstrated higher cell motility at the microgel-coated surface. These finding are in line with a considerable body of studies of biomaterial surfaces patterned in various ways, and are consistent with the concept of cell-surface interactions being regulated by the spatial distribution of cell-adhesive sites. In addition, they suggest that microgel adsorption may represent an interesting method for controlling cellular processes involved in healing after biomaterial implantation.

Similarly, Tsai et al. investigated PNIPAM microgels patterned on polystyrene substrates via dip coating [98]. Through varying substrate withdrawal speeds, surface patterns were formed, displaying stripes of densely packed PNIPAM microgels, separated by spacings contained sparsely distributed PNIPAM microgels. On cell seeding to such micropatterned substrates, NIH3T3 fibroblasts were found to adhere preferentially within the spacings to form cell patterns. Three days after seeding, cells proliferated to form confluent cell layers. The thermoresponsiveness of the underlying PNIPAM microgels was then utilized to recover fibroblast cell sheets from substrates by lowering the temperature, in analogy to the use of otherwise PNIPAM-modified or otherwise responsive surface coatings for cell sheet harvesting.

Also addressing the issue of cell interactions with different surface features of granulated microgel films, Lynch et al. investigated an approach to prepare polymeric surfaces of controlled surface topography on the micrometer scale via microgel particles [99]. Through modification of particle-particle interactions, microgels were caused to phase separate into particle-dense and particle-dilute domains, which were subsequently arrested on the surface after solvent evaporation. By changing the particle size, it was possible to alter the size of the pores formed and their distribution in the film. It was shown that such systems can be arrested into a various different structures, even formed from microgel particles of the same size and same chemical structure. As demonstrated for HeLa cells grown on 200 nm tertbutylacrylamide/isopropylacrylamide microgel surfaces, the cells can either grow inside the microgel pores, where their spreading is restricted to the size of the pore, or they can grow along the particle-rich ridges between the pores, in which case the cells adopt an elongated structure. Making controlled surface heterogeneity into a key design parameter, Li et al. combined surface-wettability-guided assembly and microdroplet-array-based operations, using polydimethyldisiloxane stamping followed by fixation, for the assembly of thousands of heterogeneous 3D cell microenvironments with precise control over individual shapes, sizes, chemical concentrations, cell density, and 3D spatial distribution of multiple components, e.g., constructed as orthogonal gradients [100].

In one of only few studies on more complete biological responses to microgel-coated surfaces, Bridges et al. investigated the biological response of thin films formed by PNIPAM microgels cross-linked with poly(ethylene glycol) diacrylate [101]. These particles were grafted to PET substrates to conformal coatings, which were found to significantly reduce fibrinogen adsorption, as well as adhesion and spreading of primary human monocyte/macrophages. The microgel coatings were also found to result in reduced leukocyte adhesion, as well as in pro-inflammatory cytokines (TNFa, IL-1b, MCP-1) after intraperitoneal implantation (Fig. 12). Taking the evaluation of the biological response to microgel-coated surfaces further to the in vivo situation, Bridges et al. investigated chronic inflammatory responses to microgel coatings consisting of PNIPAM microparticles cross-linked with poly(ethylene glycol) diacrylate deposited on PET [102]. In doing so, unmodified and microgel-coated PET disks were implanted subcutaneously in rats for 4 weeks and explants were analyzed by histology and immunohistochemistry. Microgel coatings were found to reduce chronic inflammation and to result in thinner fibrous capsules that contained 40% fewer cells compared to unmodified PET disks. Furthermore, microgel-coated samples contained significantly higher levels of macrophages (80%) than unmodified PET controls. Taken together, these results demonstrate that microgel coatings reduce chronic inflammation caused by implanted biomaterials.

There are, however, also reports of microgel coatings not succeeding to provide improved performance and biological response to biomaterials. Thus, considering that the performance of neural electrodes implanted in the brain is frequently limited by host response in the surrounding brain tissue, including astrocytic scar formation, neuronal cell death, and inflammation, Gutowski et al. investigated host responses to silicon neural electrodes with and without surface coatings formed by PNIPAM-AAc-PEG microgels [103]. In vitro, significantly reduced astrocyte and microglia adhesion were observed for the microgel-coated electrodes compared to uncoated controls. Furthermore, microgel coatings reduced astrocytic recruitment around the implant for electrodes implanted into the rat brain cortex. However, microglial response indicated persistence of inflammation, and neuronal density around the implanted electrodes was lower for both implant groups compared to the uninjured control. Taken together, it was therefore concluded that microgel coatings do not significantly improve host responses to implanted neural electrodes.

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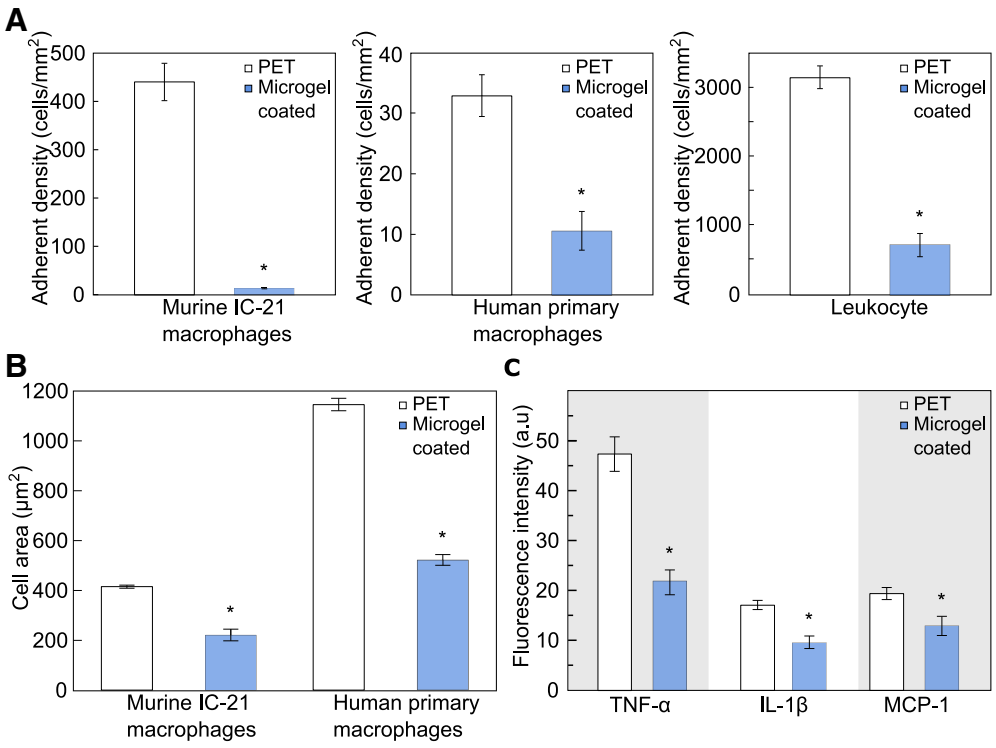

Fig. 12. Microgel-based surface modification suppresses adverse biological response to biomaterials. Surface coating of PET by PNIPAM-based microgels cross-linked by poly(ethylene glycol) diacrylate strongly reduces adhesion (a) and surface-induced deformation (b) of murine IC-21 macrophages, human primary macrophages, and leukocytes. (c) Analogously, microgel coating of PET reduces generation of pro-inflammatory cytokines.
(Re-drawn from [101].)

Although the primary focus within the context of biomaterials has been placed on implant-related issues, microgel-coated surfaces offer opportunities also in regenerative medicine, e.g., for differentiating and growing cells to confluent sheets, to be used either as biomaterials surface coatings or in regenerative medicine applications. In analogy to surfaces modified by grafting or adsorption of PNIPAM or other (thermo)responsive polymers [104], cell adhesion is good at elevated temperature (i.e., at chain collapse induced by poor solvency conditions), whereas cell detachment occurs at lowering the temperature or otherwise improving solvency for the PNIPAM chains, causing these to swell. Investigating these effects, Schmidt et al. demonstrated that PNIPAM microgel films can be used for controlled detachment of adsorbed cells via temperature stimuli [105]. In doing so, the microgel properties in the adsorbed state, as well as their changes upon temperature variation, were studied by AFM. The analysis shows that water content, surface adhesion, and nanomechanical properties all change drastically when crossing the critical temperature of the polymer film, forming the basis of the fast cell response to temperature changes, both in terms of the number of cells adhered and in their morphology. Analogous results were reported also by Uhlig et al. [106].

Within the context of bioengineering, notably polymer-based muscle expansion/contraction, Islam et al. deposited monolayer of PNIPAM-AAc microgels on an Au-coated substrate to obtain a microgel-based device able to do work in response to humidity changes [107]. Once deposited, the microgels formed a homogenous layer, the thickness of which was determined by the microgel diameter in solution. Subsequently, a solution containing oppositely charged poly(diallyldimethylammonium chloride) (PDADMAC) was added to the microgel-coated substrate and allowed to dry. Due to the strength of the PDADMAC-microgel interaction, as well as the drying-induced contraction of PDADMAC, the substrate bends on drying. An experiment was designed to investigate whether the device can be used as an artificial muscle to lift and release weight in response to humidity. To do this, weights were hung onto a curled substrate and exposed to changes in environment humidity. The device was found to open up in response to increasing humidity and to curl back up when the humidity was decreased, equivalent to an arm curling a mass with a capacity to lift 14 times the mass of the device.

Although biomaterials have attracted the most interest in relation biofouling and biological response, surface-bound microgels are of interest also for reducing biofouling in other contexts, e.g., in marine coatings. For example, Hong et al. prepared hybrid antifouling coatings with a self-generated topographical microgel surface by surface-functionalized acrylamide/methacrylic acid microgels combined with a self-peeling resin and axiridine as a polyfunctional cross-linking agent [108]. After immersion into sea water, the microgels embedded in the film surface are swollen, generating small and soft bumps. At the same time, the hydrolysis of resin makes the surface layer hydrophilic, forming a thin layer of soft hydrogel. Such a soft and dynamic surfaces are self-generating. Both the self-peeling and presence of the microgel particles result in efficient antifouling properties in real marine environment field tests.

7. Summary and outlook

Surface-bound microgels offer a wide range of opportunities for biomedical applications. As discussed in greater detail elsewhere [5], microgels in suspension are powerful delivery systems for biomacromolecular drugs, notably proteins and peptides, for which microgels may provide biological stability through preservation of secondary and tertiary structure, avoidance of aggregation, and elimination of chemical and enzymatic degradation. Furthermore, the numerous and diverse triggering factors available to such systems make them versatile tools in a host of drug delivery contexts of both biomacromolecular and low molecular weight drugs. To fully utilize these opportunities, however, further work is needed to better understand factors underlying less than ideal performance of microgels as drug delivery systems, including shell formation, incomplete drug loading, and slow/incomplete drug release.

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Another area of considerable importance concerns hybrid systems composed my microgels and inorganic nanomaterials. Of particular interest, many inorganic nanomaterials are addressable with external fields (notably light/NIR and magnetic fields). Within drug delivery, functional biomaterials, and regenerative medicine, such responsiveness provides opportunities to trigger drug release in a dose-on-demand set-up [109]. This also opens up new opportunities in theranostics, where drug-containing microgels are used for disease diagnostics, triggered drug release, and monitoring of therapeutic outcome. However, in order to be able to use such hybrid surface coatings for externally triggered drug release, for release of cell proliferation or differentiation cues in regenerative medicine, or the like, further work is needed to clarify factors determining nanoparticle interactions in such systems, e.g., relating to how localized heat or reactive oxygen species generated by the application of, e.g., light/NIR and magnetic fields, translate into swelling/deswelling transitions of the microgel network, and how this in turn may affect release of, or exposure to, bioactive cargo molecules.

As with other types biomaterials and drug delivery systems, also non-technical aspects may influence or even determine which microgel approaches will prove successful in reaching clinical trials and beyond. For example, the overwhelming majority of studies regarding surface-bound microgels have been performed with PNIPAM-based and related microgels, suffering of uncertain toxicity [110–113]. For microgels to be interesting in drug delivery, functional biomaterials, and regenerative medicine, their toxicity needs to be low in relation to the beneficial effect gained. Considering this, the scarcity of studies addressing function of surface-bound microgels in complex biological contexts, as well as in vivo toxicity, is striking. Such studies need to address triggering of the complement and coagulation systems or generation of inflammatory cytokines, but also effects on coagulation and initial cell responses. For materials intended for implantation, longer-term toxicity effects also need to be monitored, including effects on fibrous capsule formation, tissue integration, and systemic effects, in the same way as generally done for other types of biomaterials. Furthermore, while non-degradability may be acceptable in some types of implants, controlled degradation and life-time is generally required and needs to be given more consideration in relation to microgel-based biomaterials and drug delivery systems. Moreover, since the majority of studies on surface-bound microgels have been performed with PNIPAM-, PNIPAM-AAc, and related microgels, responsiveness studies have involved primarily triggering by temperature and pH. For microgels in suspension, on the other hand, considerably more specific triggers have been investigated, including triggering by reducing conditions, presence of reactive oxygen species, protein conformational changes, or presence of virtually any antigen or antibody. Analogous studies are needed to clarify to what extent similar approaches can be used to achieve triggerability of surface-bound microgels, and how this can be used in biomedical applications. Such a development is also likely to provide opportunities for microgel-based biosensing, e.g., within the context of etalons and other microgel hybrid and structured materials.

In addition, complicated designs involving multiple preparation steps (e.g., LbL multilayer build-up, complex cross-linking or grafting chemistries, and conjugation with complex and/or sensitive biological response or targeting functions) are likely to be less successful in the context of biomaterials and therapeutics development. Further attention is therefore needed on simpler and more robust approaches. As delivery systems will have to be optimized not only to the biological indication at hand, but also to the drug at hand, a considerably broader range of drugs and other types of cargo molecules also needs to be investigated in such a development.

Despite these challenges, much progress has been reached during the last decade regarding essentially all of the above issues in related areas. In particular, transfer of knowledge generated for microgel suspensions to surface-bound microgels is expected to be a very fruitful area research and development during the coming years.

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