

Polymer Communication
Bilayer hydrogel assembly

Dirk Kuckling*, Pradeep Pareek

Fachrichtung Chemie und Lebensmittelchemie, Technische Universität Dresden, D-01062 Dresden, Germany

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Abstract

The combination of two hydrogel layers based on photo-cross-linkable poly(dimethyl acrylamide) (PDMAAm) as base layer and poly(*N*-isopropyl acrylamide) (PNIPAAm) as top layer forms a bilayer assembly where, the base layer is highly swollen and the top layer shows temperature responsive swelling. Characterization was done by a combination of surface plasmon resonance spectroscopy and optical waveguide spectroscopy as well as atomic force microscopy. The PDMAAm and PNIPAAm within the bilayer assembly retain their swelling behavior, which they showed as a separate layer. Due to the presence of the photo-cross-linker such bilayer films can be patterned to develop surfaces with different properties at different regions.

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The volume phase transition in stimuli-sensitive hydrogels is important for many applications, e.g. as (micro-)actuator and sensor materials [1], or in controlled cell attachment–detachment [2,3] and controlled drug delivery [4]. Most investigations focus on temperature or pH sensitive polymers, however, a variety of other parameters (e.g. ionic strength, UV light, magnetic fields, etc.) have been studied [5]. The majority of these applications require the use of hydrogels at surfaces and interfaces. Therefore, the behavior of bulk hydrogel may not be necessarily extended to these types of geometries. Multilayers of polymers are of interest due to their diverse applications, e.g. as biomaterials [6,7]. Alternate coating of oppositely charged films produces polyelectrolyte multilayers [8]. In such cases, application relevant properties like switchability and patternability are rarely observed. Heterogeneous mixed polymer brushes (HPB) have the ability to respond in a controllable fashion to specific environmental stimuli. Recently, HPB of poly(styrene) and poly(2-vinylpyridine) have been reported. Using such technique, surface property can be changed in the presence of specific solvents [9,10]. Switching mechanism of these brushes involves use of organic

solvents, which are not suitable for bio-applications. Multilayers of hydrogels with patternable properties can provide a surface with distinct behavior at different regions of the surface in the presence of a stimulus. A schematic presentation of a possible assembly is given in Fig. 1.

In order to study the bilayer assembly photo-cross-linkable poly(*N*-isopropyl acrylamide) (PNIPAAm) was chosen as a stimuli-sensitive component and poly(dimethyl acrylamide) (PDMAAm) as a hydrophilic component. Film thickness of both the layers could be controlled either by polymer solution concentration or by spin speed in coating. Synthesized polymers have predefined properties like molecular weight and photo-cross-linker content, which result in the uniform cross-linking of the thin film.

Photo-cross-linker (2-dimethylmaleimido) ethylacrylamide (DMIAAm) was synthesized in a four-step process [11]. Photo-cross-linkable polymers of DMAAm were obtained by the copolymerization of DMAAm with DMIAAm in different feed mol% of these monomers. DMIAAm content is indicated as suffix behind the respective monomer (e.g. PDMAAm5). Molecular weights (M_w) of the copolymers were in the range of 51,000–71,000 g/mol. Photo-cross-linkable polymer of NIPAAm was obtained by copolymerization of NIPAAm, DMAAm and DMIAAm in the feed mol% ratio 80/15/5 of

* Corresponding author. Tel.: +49 351 463 33788; fax: +49 351 463 37122.
E-mail address: dirk.kuckling@chemie.tu-dresden.de (D. Kuckling).

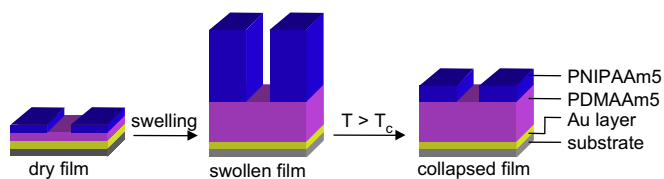


Fig. 1. Schematic representation of a patterned photo-cross-linked bilayer assembly with bottom layer as a hydrophilic layer and top layer as a stimuli-sensitive layer.

these monomers (abbreviated as PNIPAAm5) [12]. Surface attached hydrogel layer of DMAAm polymer was prepared using an adhesion promoter [13].

PDMAAm5 polymer solution (5 wt% in cyclohexanone) was spin coated at 3000 rpm for 180 s on a SPR substrate (LaSFN9 glass, 45 nm gold and adhesion promoter). After vacuum drying at room temperature for 1 h, the thin film was photo-cross-linked by UV irradiation for 1 h. This provided a cross-linked insoluble network of PDMAAm, which was used as a solid substrate for coating the second layer. PNIPAAm5 polymer solution (5 wt% in cyclohexanone) was spin coated on top of the PDMAAm5 thin film under the same conditions and photo-cross-linked by UV irradiation for 1 h. Cross-linking was achieved by [2 + 2] cyclodimerization of the dimethylmaleimide moiety present in the polymer chains. In order to achieve linkage of the two hydrogel layers it is important to choose a solvent for spin coating of the top layer that is a good solvent for the first gel as well. During film formation a thin interpenetrating network is formed and afterwards fixed by UV irradiation. Selection of a poor solvent did not yield in entanglement and, thus, in bilayer formation.

A combination of surface plasmon resonance spectroscopy (SPR) and optical waveguide spectroscopy (OWS) [14] was used to determine the refractive index, film thickness and swelling behavior of thin photo-cross-linked hydrogel films (for details see Ref. [15]). The principle is based on the detection of refractive index changes in a thin dielectric layer on top of a metal surface and probed by the evanescent field of a laser beam. The reflected intensity of the beam is recorded as a function of incident angle and it decreases dramatically as the light couples into the plasmon mode of the metal or the waveguide of the dielectric. The evanescent wave of the plasmon decays exponentially into the dielectric and therefore it makes SPR as a surface sensitive technique. For the dielectric with known refractive index, this technique provides information about the film thickness or vice versa.

The same set up was used for the determination of film thickness in the case of thick films (dry thickness > 500 nm) by optical waveguide spectroscopy. For thicker films the position of the plasmon minima is sensitive only to the refractive index while the position of waveguide modes depends on both film thickness and refractive index. OWS provides more accurate results, as film thickness and refractive index could be determined independently. In the following the combination of SPR and OWS measurements will be referred as SPR/OWS measurements. The refractive index of phosphate buffered saline (PBS buffer) solution is temperature dependent and was taken into account for evaluation of the data.

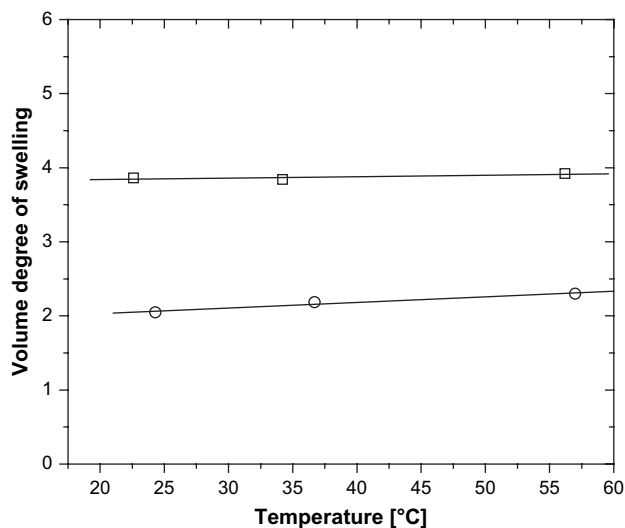


Fig. 2. Volume degree of swelling ($1/\phi_p$) of PDMAAm photo-cross-linked hydrogel layers (■ – PDMAAm2; ○ – PDMAAm5).

First the swelling behavior of single photo-cross-linked PDMAAm hydrogel layers with varying mol% of acrylamide photo-cross-linker (DMAAm) was measured in PBS buffer. The DMAAm homo- and copolymers are soluble in common organic solvent and hence are suitable for homogeneous thin film formation. It is known that the DMAAm polymers are hydrophilic, but not thermo-responsive. The swelling behavior of photo-cross-linked DMAAm hydrogel investigated by SPR/OWS is shown in Fig. 2. The refractive index of the hydrogel layer obtained from SPR/OWS was converted to the polymer fraction (ϕ_p) and volume degree of swelling ($1/\phi_p$) [13].

Notable features observed from Fig. 2 are that the swelling behavior of PDMAAm photo-cross-linked hydrogels is almost independent of the temperature changes. PDMAAm2 exhibits refractive indices of $\eta_{\text{dry}} = 1.473$ and $\eta_{\text{swollen}} = 1.380$, respectively, corresponding to a volume degree of swelling ($1/\phi_p$) of 3.8 between 22.6 °C and 56.2 °C. However, PDMAAm5 exhibits refractive indices of $\eta_{\text{dry}} = 1.476$ and $\eta_{\text{swollen}} = 1.424$, respectively, corresponding to $1/\phi_p$ of 2.0–2.2 between 24.3 °C and 57 °C. This clearly suggests that the swelling in photo-cross-linked PDMAAm hydrogel is temperature independent, however, it depends on the mol% of the photo-cross-linker. An increase in the photo-cross-linker content resulted in decrease in the volume degree of swelling. Such polymers will be used as a base layer in the bilayer hydrogel assembly. The swelling behavior of PNIPAAm5 has been described previously [12].

Bilayer swelling involving a stimuli-sensitive layer and a hydrophilic layer was measured in the dry state as well as in PBS buffer. The SPR/OWS angle scans of this bilayer hydrogel film in dry state are shown in Fig. 3. PDMAAm5 layer in absence of PNIPAAm5 layer showed one waveguide mode at 35.8°. Spin coating and the subsequent cross-linking of PNIPAAm5 layer on PDMAAm5 layer showed two waveguide modes at 27.0° and 51.1°. The presence of a clear total internal reflection (around 22.3°) and narrow waveguide modes indicated the formation of homogeneous bilayer films.

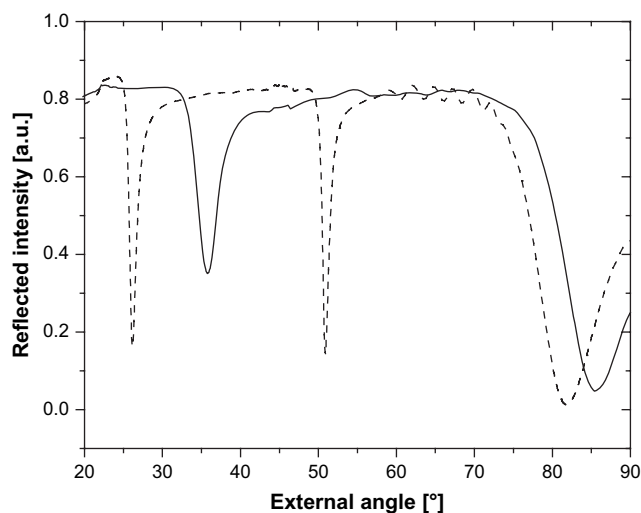


Fig. 3. SPR/OWS angle scans of the photo-cross-linked bilayer assembly in dry state (solid line – PDMAAm5; dashed line – bilayer of PDMAAm5 and PNIPAAm5).

SPR/OWS angular scans obtained for a bilayer hydrogel assembly involving PDMAAm5 as a hydrophilic base layer and PNIPAAm5 as a stimuli-sensitive layer were analyzed by a box model. A two-layer model was used to investigate the properties of bilayer hydrogel films like film thickness, refractive index and volume degree of swelling for both the layers. For such a bilayer, the SPR/OWS angular scans were also modeled as a single layer with one refractive index. However, the results were not consistent with the experimental values. Therefore, a two-layer model was used to describe the bilayer hydrogel assembly. The refractive index of the two films was entered separately. Plasmon minima and both waveguide modes from SPR/OWS angular scans are in good agreement with this model. The thickness of the dry film could also be determined by the appearance of waveguide modes. The determined value of PDMAAm5 layer thickness was 372 nm and bilayer thickness was 602 nm.

The reflected intensity vs. angle scans of bilayer assembly in PBS buffer at various temperatures was analyzed. The refractive indices of bilayer hydrogels obtained from SPR/OWS were converted to polymer fraction (ϕ_p) and volume degree of swelling ($1/\phi_p$). Fig. 4 describes the changes in refractive index (η) and volume degree of swelling of the bilayer hydrogel in response to the temperature changes.

PNIPAAm5 layer in swollen state showed $\eta_{\text{swollen}} = 1.368$ with a volume degree of swelling $1/\phi_p = 5.1$ and its collapsed state showed $\eta_{\text{collapsed}} = 1.428$ with $1/\phi_p = 1.9$. The transition temperature (T_c) was determined to be 34.7 °C. PNIPAAm5 layer when directly attached to gold surface in the absence of a base layer showed $1/\phi_p = 5.4$ and its collapsed state showed $1/\phi_p = 1.6$. The transition temperature was determined to be 34.1 °C [12]. On the basis of these observations, it is clear that PNIPAAm5, which forms the top layer in the bilayer hydrogel assembly, retains its temperature sensitive behavior. It exhibits a T_c and $1/\phi_p$ similar to that of PNIPAAm5 single layer. A small difference in the swelling behavior of

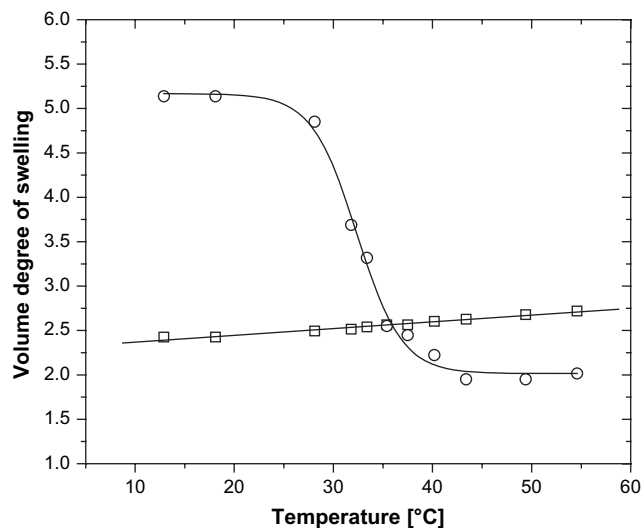
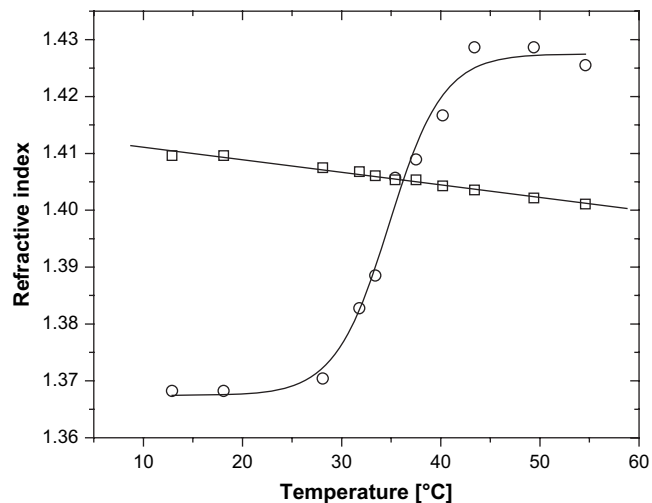


Fig. 4. Refractive index (upper diagram) and volume degree of swelling (lower diagram) of the photo-cross-linked bilayer as a function of temperature in PBS buffer (■ – PDMAAm5 layer, ○ – PNIPAAm5 layer).

PNIPAAm5 in the bilayer hydrogel assembly and in the single layer is quite reasonable. This can be explained on the basis that the morphology of the film and its thickness are affected by the surface energy of the substrate due to the segregation or depletion of different components at the interface [16] or dewetting of the film below a certain thickness [17]. Therefore, the interfacial energy between the gold–polymer in the single layer and polymer–polymer in the bilayer assembly might give a small difference in the swelling behavior of the two systems. These differences are small and can be neglected in most applications.

PDMAAm5 as base layer of the bilayer assembly showed an almost constant η_{swollen} between 1.409 and 1.401, with $1/\phi_p = 2.4$ – 2.7 in response to temperature changes. This indicates a temperature independent swelling for the hydrophilic base layer. PDMAAm5 as a single layer on gold shows $\eta_{\text{swollen}} = 1.411$ and $1/\phi_p = 2.3$. These results confirm that the PDMAAm5 and PNIPAAm5 in bilayer assembly retain their swelling behavior, which they showed as a separate single layer.

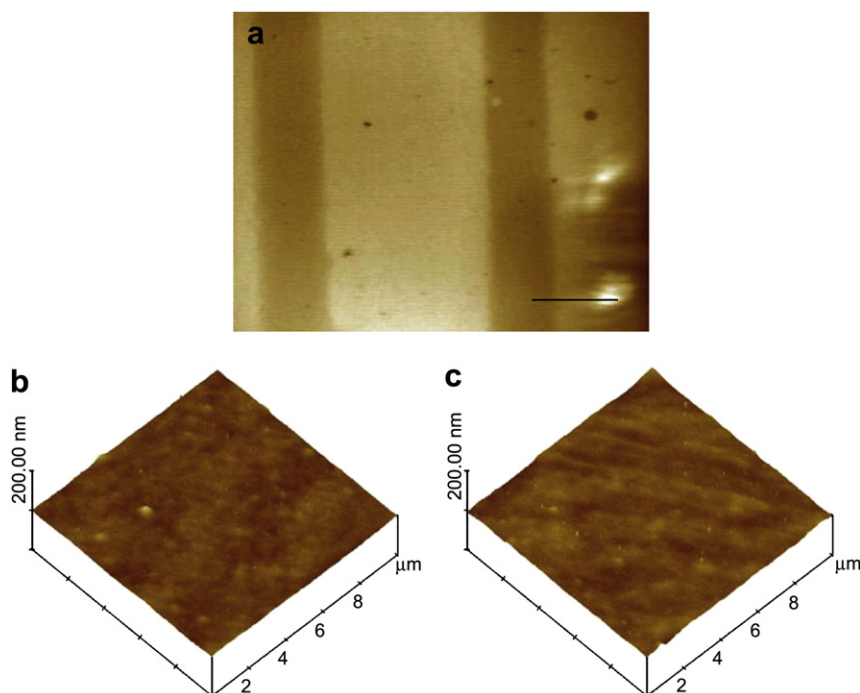


Fig. 5. AFM images of patterned bilayer of PDMAAm5 and PNIPAAm5 assemblies in the dry state (scale bar 100 μm): topology (a) as well as details of PNIPAAm5 (b) and PDMAAm5 (c) surfaces.

Due to the presence of photo-cross-linker such multilayer films can be patterned to develop surfaces with different properties at different regions. The possibility to pattern photo-cross-linkable polymers of PNIPAAm and PDMAAm makes them useful for application in micro-system technology [18,19]. Patterned thin layers were obtained by irradiating a polymer film with UV light through a common chromium mask on both Au and Si wafers. The photo-patterning turned out as a negative photo-lithographic process. Hydrogel was formed at the UV exposed areas, leaving uncross-linked polymer at unexposed areas. It was observed that the cross-link density significantly affects the resolution properties of the patterned surfaces. This implies that a loosely cross-linked network obtained by irradiating the film for 1–5 min contains a low cross-link density and therefore results in highly swollen hydrogel. As a result of this behavior, the hydrogel film was prone to detach from the substrate producing patterns with some defects.

In order to obtain patterned hydrogel films, first PDMAAm5 was spin coated onto pretreated Si wafers and cross-linked. This was followed by spin coating of PNIPAAm5 and subsequent photo-cross-linking through a mask. Irradiation time of 1 h resulted in formation of patterns with high resolution. The darker areas in Fig. 5 (higher in topography) correspond to the PNIPAAm5 hydrogel and brighter areas (lower in topography) correspond to PDMAAm5 hydrogel.

Tapping mode atomic force microscopy (AFM) was used to characterize the surface topography of the patterned bilayer films. The root-mean-square roughness of PNIPAAm5 hydrogel top layer and PDMAAm5 hydrogel base layer in dry state were determined to be 1.2 nm and 1.7 nm, respectively. This implies formation of a uniform and continuous pattern on

the bilayer hydrogel assembly. The height difference between the two hydrogel layers was measured to be 23 nm in the dry state corresponding with the expected film thickness of the PNIPAAm5 hydrogel layer. The AFM studies clearly indicate the presence of two hydrogel layers.

In conclusion, hydrogel multilayers can be prepared from photo-cross-linkable polymers by successive deposition using a proper solvent. Within the multilayer hydrogel assembly, chemically different hydrogel layers retained their swelling properties in response to temperature changes. Different photo-cross-linked hydrogels in a multilayer assembly have shown high resolution patterns and uniform film formation.

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