Surface Chemistry

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Mechanically Induced Generation of Counterions Inside Surface-Grafted Charged Macromolecular Films: Towards Enhanced Mechanotransduction in Artificial Systems**

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Mechanical stresses modulate a variety of physiological functions, ranging from the detection of sound (hearing) to the growth of bone tissue. Physical forces or strains can activate mechanosensitive ion channels at the cell surface, alter the conformation of the extracellular matrix, or alter the function of tension-sensitive components of the cytoskeleton.[1] There is a significant research effort to discover how external mechanical forces regulate cell activity: from distortions of the cytoskeleton to the activation of gene expression and the synthesis of proteins.[2]

It is well-known that highly charged environments play an important role in the mechanical properties of biological tissues,[3,4] as is the case in articular cartilage where the compressive resistance is mainly due to electrostatic repulsive forces at the molecular level between highly charged chondroitin sulfate glycosaminoglycan chains.[5–8] The fundamental question central to the work reported here is if mechanical pathways can be exploited for manipulating chemical reactions[9,10] in confined environments and in particular how mechanical stresses can influence the chemical properties of thin polyelectrolyte films.

This initial study on model macromolecular systems to convert physical forces into chemical information aims to provide a design template for "smart" thin films whose response can be triggered by external mechanical stimuli.

Our thin polymer films consist of polyelectrolyte brushes, that is, charged polymers tethered at one end to the surface. At sufficiently high grafting densities in pure water the polymer chains are stretched away from the surface as a result of strong repulsion between the charges on the monomers. However, the addition of an electrolyte leads to screening of these charges and, as a consequence, to the collapse of the brushes.[11,12]

In seminal work, Klein and co-workers have shown that two surfaces coated with strong polyelectrolyte brushes exhibited significantly improved lubrication because of resistance of the brushes towards collapse.[13] The crucial observation we report here is that deliberate mechanical compression of strongly charged polyelectrolyte brushes in pure water should lead to a state where the microenvironment in the brush layer resembles the high ionic strength environment that would otherwise lead to collapse so as to minimize Coulombic repulsions between the charges on the brushes. To put this idea into practice we exploited the ionization of dye molecules immobilized inside polyelectrolyte brushes upon mechanical deformation of the surface-grafted polymer chains.

Polyelectrolyte brushes based on poly([2-methacroyloxy)ethyl]trimethylammonium chloride) (PMETAC; Figure 1) were synthesized using surface-initiated aqueous atom transfer radical polymerization (ATRP) from silane-initiator-modified glass slides following a previously reported procedure.[14–16] All the experimental details are described in the Supporting Information.

The brushes ($h \approx 20$ nm, "dry" thickness) were washed thoroughly with Milli-Q water to remove any excess salts, and subsequently placed in a 0.6 mM aqueous solution of bromothymol blue (BTB). BTB is known as a highly sensitive probe to monitor chemical and physical changes in biomembranes and proteins.[17] This dye changes its color at around pH 7, from a yellow (neutral form) to a blue (anionic form) state, which can be easily detected by UV/Vis spectroscopy.[18,19] Mechanical compression was accomplished by sandwiching the brush-coated glass slides between two slabs of cross-linked poly(dimethylsiloxane) (PDMS) and placing the assembly in a holder commonly used for IR experiments on liquids. Great care was taken to ensure that the brush-coated glass slides were never dried, since dehydration would inevitably lead to (irreversible) collapse of the brushes. The use of elastomeric PDMS is essential to ensure conformal contact with the brush surface and a homogeneous distribution of the load.[20,21] The screws on the cell were tightened to place the whole assembly under compression (up to approximately 0.1 MPa). Figure 2 shows the changes detected in the UV/Vis absorption spectra upon compression of the PMETAC/BTB assembly. The spectrum of BTB with no pressure applied shows the equilibrium population of both anionic and neutral species inside the hydrated (pH 7) brush.

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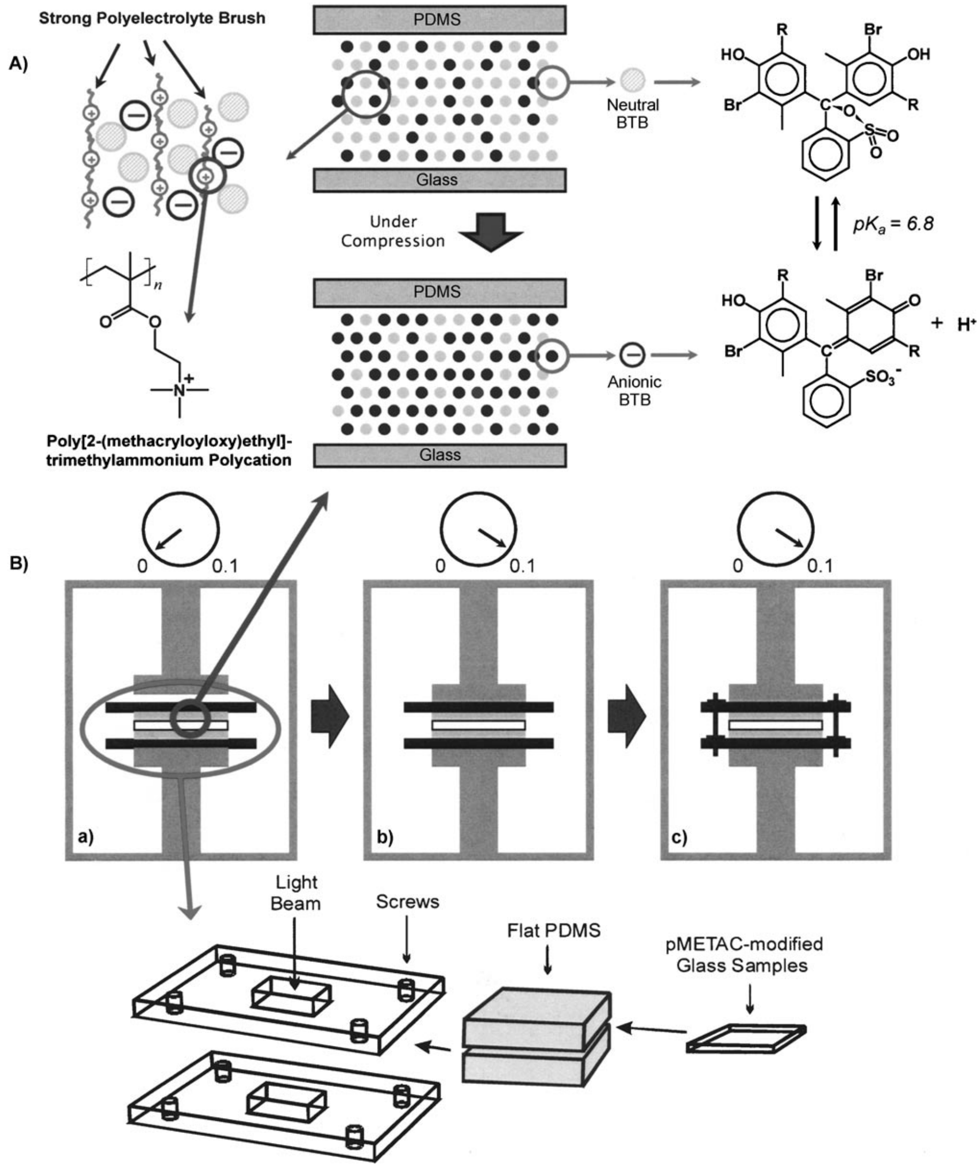

Figure 1. A) Simplified depiction of the variation in the population of neutral (light circles) and anionic (dark circles) bromothymol blue (BTB) species inside PMETAC brush films upon compression. Also depicted in the schematic representation are the chemical structures corresponding to the cationic polyelectrolyte brush (left) and the neutral and anionic form of the BTB dye (right). B) Schematic representation of the experimental setup used to compress the polyelectrolyte brushes. The IR cell containing the PDMS-sandwiched PMETAC-coated glass was introduced into a small laboratory press (a). The sandwiched glass slide was compressed at 0.1 MPa (b). Thereafter, the sandwiched experimental arrangement was fixed under compression using screws (c). The bottom part of the Figure depicts in detail the sandwiched glass slide introduced in the IR cell.

It should be noted that the strongly charged environment of the brush always leads to a certain additional ionization of BTB, partly as a result of the formation of a complex between the quaternary ammonium (QA*) groups and the sulfonate moieties in the dye molecules.[22]

However, a significant and easily detectable increase in the absorption at 625 nm (anionic form) was clearly observed when pressure was applied (Figure 2a). This change was accompanied by a decrease in the absorption at 430 nm (neutral form).

A similar trend upon compression is observed when starting from a different population distribution of anionic and neutral species (namely, placing the dye-loaded brushes in a solution at a different pH value). Compressing the BTB-loaded hydrated PMETAC brush at pH 4 again evidences the enhancement of the anionic form at 625 nm, together with a decrease in the absorption of the protonated dye at 430 nm (Figure 2b).

This increase in the absorption at 625 nm upon compression is clearly related to an increase in the population of anionic BTB dye molecules inside the brush layer. In strong polyelectrolyte brushes, the charges strongly repel each other in pure water and mechanical compression of such brushes would not be possible, since the repulsive Coulombic interactions cannot be easily overcome. In our case, with the dye added to the brush layer, the mechanical stresses effectively result in a pressure-induced Coulombic imbalance, and the excess of repulsive Coulombic interactions are rapidly compensated and effectively screened through an increase in the population of counterions resulting from dissociation of the dye (Figure 2). In other words, the presence of the dye molecules allows the brushes "an easy way out", and instead of resisting mechanical compression, the brushes can now collapse in response to mechanical stress. From a chemistry point of view, the mechanical stress combined with the nanoconfinement inside the brush layer affects the chemical equilibrium of the BTB inside the brush and as a consequence its pK_a value. In the absence of any pressure the equilibrium situation can be simply described as Equation (1).

$$\begin{array}{llll} \mathrm{BTB} \rightarrow & \mathrm{BTB}^- + & \mathrm{H}^+ & K_a = \frac{[\mathrm{H}^+][\mathrm{BTB}^-]}{[\mathrm{BTB}]} = \frac{C^2\alpha^2}{C(1-\alpha)} \\ C(1-\alpha) & C\alpha & C\alpha & \end{array} \quad (1)$$

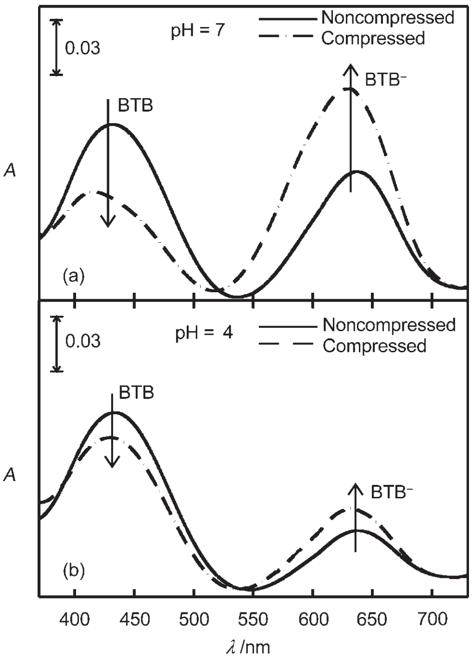

Figure 2. UV/Vis spectra corresponding to the compression experiments for BTB-loaded PMETAC brushes in a hydrated environment at different pH values: a) pH 7 and b) pH 4. Dotted and solid lines refer to spectra taken under compression and with no compression, respectively. The arrows indicate the direction of changes upon compression.

Here, K_a is the dissociation constant of the BTB dye, C is the formal concentration of the dye inside the brush, and α is the degree of dissociation at zero pressure. At 0.1 MPa the same equilibrium can be described as Equation (2),

$$\begin{array}{lll} \mathrm{BTB} \rightarrow & \mathrm{BTB}^- + \mathrm{H}^+ & K_\mathrm{a}^{0.1\,\mathrm{MPa}} = \dfrac{[\mathrm{H}^+][\mathrm{BTB}^-]}{[\mathrm{BTB}]} = \dfrac{C^2\beta^2}{C(1-\beta)} \\ C(1-\beta) & C\beta \quad\quad C\beta & \end{array} \tag{2}$$

where $K_\mathrm{a}^{0.1\,\mathrm{MPa}}$ and β are the dissociation constant and the degree of dissociation at 0.1 MPa, respectively.

Considering that a) the formal concentration (C) of the dye inside the brush remains the same, independent of pressure, and b) the concentration of the anionic dye species is approximately twice the original concentration upon compression, as derived from the change in the absorption of the anionic species at 625 nm (Figure 2a), $\beta \approx 2\alpha$ [Eq. (3)].

$$\frac{K_\mathrm{a}}{K_\mathrm{a}^{0.1\,\mathrm{MPa}}} = \frac{C^2\alpha^2}{C(1-\alpha)}\frac{C(1-\beta)}{C^2\beta^2} \approx \frac{C^2\alpha^2}{C(1-\alpha)}\frac{C(1-2\alpha)}{C^2(2\alpha^2)} \tag{3}$$

The UV/Vis spectra under zero pressure (Figure 2a) shows the distribution of species inside the brush under this condition. From the extinction coefficients (ε) of the neutral ($\varepsilon^\mathrm{N} = 1.82 \times 10^4\,\mathrm{M}^{-1}\mathrm{cm}^{-1}$) and anionic ($\varepsilon^\mathrm{A} = 3.75 \times 10^4\,\mathrm{M}^{-1}\mathrm{cm}^{-1}$) species in solution we estimated a α value of approximately 0.23. In this case Equation (3) becomes Equation (4).

$$K_\mathrm{a}^{0.1\,\mathrm{MPa}} = 5.9\,K_\mathrm{a} \tag{4}$$

Equation (4) can also be expressed in terms of pK_a values [Eq. (5)].

$$\begin{aligned} &-\log K_\mathrm{a}^{0.1\,\mathrm{MPa}} = -\log(5.9\,K_\mathrm{a}) = -[\log 5.9 + \log K_\mathrm{a}] \\ &= \mathrm{p}K_{\mathrm{a}_\mathrm{a}}^{0.1\,\mathrm{MPa}} = \mathrm{p}K_\mathrm{a} - \log 5.9 = \mathrm{p}K_\mathrm{a} - 0.77 \end{aligned} \tag{5}$$

This equation shows that the compression of the brush was transducted in a shift in the pK_a value of about 0.77, as derived from the increase in the population of the anionic form of the BTB dye. Further confinement of the macromolecular layer promotes an increase in the electrostatic repulsion between the QA$^+$ moieties thus triggering the formation of new ion pairs to overcome the Coulombic unbalance.

The change in absorption upon compression is irreversible; once the brushes collapse, the dye molecules do not revert to their precompression equilibrium. To explore the origin of the irreversibility we studied the conformational changes (brush height, h) occurring in the polymer layer prior to and after compression by using atomic force microscopy (AFM). To estimate h under different conditions we have performed AFM measurements on patterned PMETAC brushes in water (Figure 3a). By combining AFM imaging with patterned responsive brushes in liquids it is possible to obtain reliable information about the actual h value of the polymer brush in the liquid environment.[15,16] As expected, PMETAC when imaged in pure water shows an extended conformation ($h \approx 60$ nm) as a result of the strong repulsion between the like-charge monomers. Conversely, the dye-loaded PMETAC presents a less-extended conformation ($h \approx 45$ nm) when imaged under similar conditions. This fact is in agreement with the strong association (ion pairing) of the BTB dye with the cationic brush. After compressing the patterned samples, AFM of the samples in water revealed that

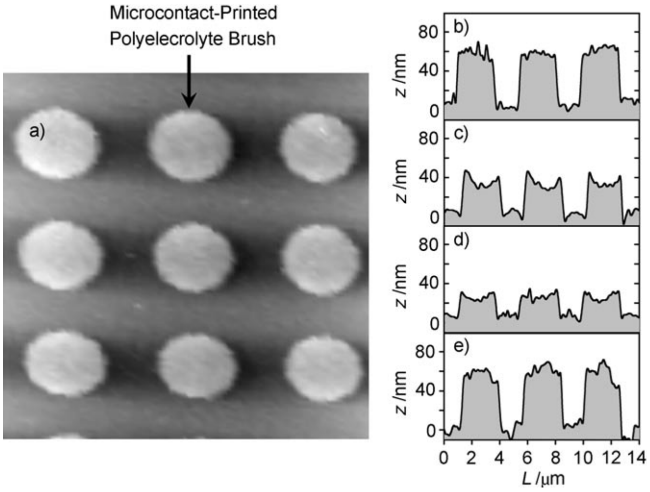

Figure 3. a) AFM image (14×14 µm^2) of patterned PMETAC brushes (dot shaped) on a gold substrate. Cross-sectional analyses derived from AFM imaging in water corresponding to: b) as-synthesized PMETAC brushes, c) BTB-loaded PMETAC brushes, d) BTB-loaded PMETAC brushes after compression, and e) as-synthesized PMETAC brushes after compression.

the PMETAC brushes remain in a collapsed state ($h \approx 25$ nm). These results clearly support the irreversibility observed in the UV/Vis measurements. Deliberate compression triggers the formation of more ion pairs between the QA^+ moieties in the brushes and the SO_3^- groups of the dye. As previously reported in the literature, the formation of strong ion pairs is not easily reversed and the brushes remain in a strongly collapsed conformation.[15,23] This is the origin of the irreversibility observed in the system during the AFM and UV/Vis experiments. It must be noted that we carried out control experiments using PMETAC brushes without any dye. In these cases the brushes did revert to their original height after compression with a PDMS stamp (Figure 3e).

In conclusion, we have shown how mechanical forces can be channelled into shifting chemical equilibria by exploiting the properties of polyelectrolyte brushes. This approach is not limited to surfaces and could in principle be achieved in macroscopic systems, provided that nanoconfinement and compression of the polyelectrolytes can be ensured. This simple model, based on strong ion-pairing interactions of a dye probe within a surface-grafted charged macromolecular film, provides evidence that physical forces can be easily mechanotransducted into chemical signals. We believe that these results pave the way for the design of artificial (man-made) systems with mechanotransduction properties suitable for the fabrication of very sensitive and low-cost smart pressure-sensing devices involving simple read-out systems.

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