J|A|C|S COMMUNICATIONS

Published on Web 12/29/2006

Reversible Cell Deformation by a Polymeric Actuator

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Physical forces acting on cells are transduced into biochemical responses and play critical roles in cell development, migration, and morphology.[1] The effects exerted by such forces are being addressed by several techniques such as manipulation by laser tweezers[2] and use of deformable substrates.[3]

Smart materials serving as actuators in micro- and nanosystems for manipulation of structure and function of biomolecules and cells are indispensable tools in diverse biological and therapeutic applications. One such material is the thermosensitive polymer, poly (*N*-isopropylacrylamide), PNIPAM, which is studied and employed extensively in bio- and nanorelated fields.[4] PNIPAM gels undergo reversible shrinking and swelling, as well as alterations in hydrophobicity, in response to temperature changes. We exploited these properties by using PNIPAM gels as versatile actuating devices for inducing shape deformations of cells by both stretching and compression. Highly deformable red blood cells (RBC) were selected as a model system. The deformability of RBCs, the major cellular constituents of blood, has a major influence on blood flow;[5] it follows that RBC rheology is a key element in the etiology of various disease states.[6,7]

Our first approach to devising PNIPAM gel actuators was to prepare a gel on a coverslip in the presence of cells. This procedure resulted in a thin-gel layer in which RBCs were predominantly embedded between the surfaces of the gel and the glass. The thin gel layer permitted imaging by transillumination even at temperatures exceeding the lower critical solution temperature (LCST, ~32 °C), under which condition PNIPAM gels become opaque. Upon heating the sample above the LCST, the gel expelled water and contracted owing to the hydrophobically driven association of the constituent polymer chains. The forces associated with the contraction were relayed to the embedded cells, which as a result sickled (Figure 1A).

In the case of flaccid RBCs, the surface-to-volume ratio sets constraints on the degree of cell deformation.[5] Consequently, an RBC is easily deformed as long as the surface area is not obliged to increase or the cytoplasmic volume to decrease. Thus, above the LCST, the gel "pulled" the RBC such that more of the cell extended along the axial direction (not perceived in Figure 1A). Cooling the sample below the LCST caused the gel to reswell, and the cells recovered their original shape. The temperature effects on water transport may not be intuitive: below the LCST the polymer is solvated with water and hinders the flow of the mobile phase, while above the LCST desolvation of the polymer actually enables rapid solvent flow in the system.[8] Since the deformation of the RBCs occurred above the LCST, it is unlikely that the expulsion of water from the gel resulted in a pressure buildup contributing to cell deformation.

RBCs incorporated into a PNIPAM gel are randomly distributed and thus experience diverse deformation forces dependent on their orientation to the underlying surface. In addition, the chemical reagents involved in the polymerization may be deleterious to the cells. We therefore devised actuators with simpler geometries that could be applied externally to cells. The first external actuator consisted of a thin, planar PNIPAM gel layer prepared on a coverslip. Such a gel actuator can be used on cells in suspension or adhering to a substrate. In one such experiment, cells were sandwiched between the swollen gel and a glass slide. The gel exerted an effect on those cells in close proximity. Nonadherent cells were confined and thus were unable to "escape" via solution flow induced by temperature changes (Figure 1B). The application of the gel on top of the cells resulted in the formation of depressions in which several cells (in suspension) packed together (Figure 1F,H). Once the gel was in contact with the cells, the temperature was raised above the LCST. The gel became more hydrophobic,

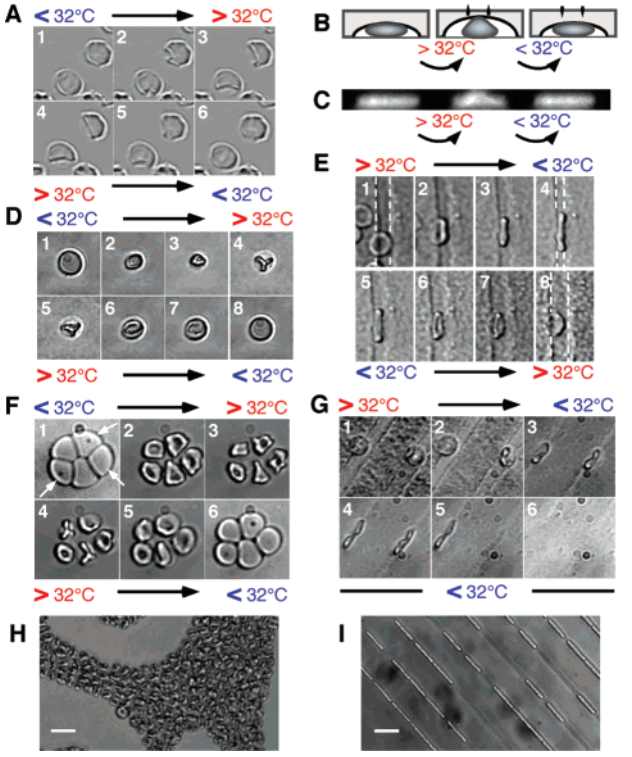

Figure 1. (A) Deformation of RBCs embedded in a PNIPAM gel. The axial direction is normal to the focal plane. Suggested mechanism (B) and side view of a fluorescently labeled RBC (C) deformed by the planar actuator. Flaccid RBC deformation by planar (D) and patterned (E) actuators (1,4,8: edges of the channel highlighted). (F) Deformation by a planar actuator of flaccid and rigid RBCs. Arrows point to rigid RBCs. (G) Rigid RBCs deformed and ruptured by a patterned actuator. Deformation of a large number of RBCs by (H) planar and (I) patterned actuators. Scale bars, 10 μm.

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10.1021/ja067171+ CCC: $37.00

promoting adherence to the cell membrane, and also assumed a more compact form. Atomic force microscope measurements of the adhesive interactions between fibronectin[9] or bovine serum albumin[10] with grafted PNIPAM surfaces have revealed that the forces required to detach proteins from the PNIPAM above the LCST are in the nanonewton range. Although likely to have different values for the different constituents of the cell membrane, these adhesive forces are much greater than the piconewtons required to stretch erythrocytes in optical tweezers experiments.[11] It was therefore expected that the contracting gel would be able to pull and deform red blood cells adsorbed on glass. From the sequence of side views of a fluorescently labeled RBC below and above the LCST (Figure 1C), we conclude that the phenomenon described above was indeed operative, causing a retraction of the cell membrane adherent to the polymer gel and deforming the cell (Figure 1B,C). Upon reducing the temperature below the LCST, the gel regained its hydrophilic character and swelled, and stress was relieved as a consequence of its diminished affinity for the cell membrane.

Figure 1D shows the reversible pulling effect of the actuated gel on the shape of an RBC. Upon heating, the RBC membrane adhered to the gel and was pulled. The sequence of shapes shown in Figure 1D suggests the existence of an intricate mechanism by which an RBC rearranges its form, leading to such an intriguing final shape. According to laser tweezers experiments and computer simulations by Dao et al.,[11] the folding of the RBC is influenced by the geometry of the contacts between beads used and the cell and by the applied stretching force. We observe that upon temperature-induced pulling of the gel, which confers a greater freedom of motion on the cells, the gel-RBC contacts readjust continuously, thus affecting cellular shape. Local differences in the adhesion of the outer surface of the cell to the gel, coupled with cellular motion, lead to a restructuring of the RBC membrane. The outcome of the adsorption and/or desorption of different molecular constituents of the membrane to and from the gel, operating under the constraints of invariant surface area and cytoplasmic volume, is to generate an energetically more favorable shape. In our experiment, the gel swelled upon lowering the temperature below the LCST and with the reduction in cell−gel affinity, the RBC regained its initial state, that is, confined within a depression (Figure 1D, panel 8).

A second external actuator was fabricated by molding PNIPAM into a patterned gel (Figure 1E). The mold was selected so as to achieve a patterned gel consisting of rectangular grooves ~6 μm wide and 1.5 μm deep in the collapsed state. Cells were placed between the shrunken patterned gel and a glass slide. Above the LCST, the gel collapsed, and thus the channels were "open". As depicted in Figure 1E, lowering the temperature below the LCST led to expansion of the gel, thereby narrowing the channels and compressing the cells residing within or near them (Figure 1E, panel 4). Heating the sample above the LCST caused the gel to retract, thus opening the channels and relieving the pressure on the cells, which returned to their original shape (Figure 1E, panels 1 and 8). In our system, the value of deformation (elongation) of the cell, defined as the difference between the length and width of the deformed cell divided by their corresponding sum, was ~0.6. Achieving such an elongation in viscometer based experiments requires shear stress in excess of 25 Pa.[12]

To study the effect of such actuators on different cells, we probed a mixture of normal and heat-treated, rigid RBCs. Heating RBCs causes physical and chemical changes in the plasma membrane that increase the viscosity, rigidity, and fragility of the cells.[13] A first intriguing observation gained with the thin layer gel actuator, was that flattening rigid RBCs almost invariably resulted in the appearance of a dimple on the RBC surface, in contrast to the smooth surface of flattened flaccid RBCs (Figure 1F). Once the temperature was elevated above the LCST, the rigid cells were much less deformable than the flaccid ones (Figure 1F, panel 4). The low deformability of rigid cells can be attributed to their altered viscoelasticity, acting to inhibit rearrangement of the cell membrane. This, in turn, affects the mobility of the cell−gel contact points reacting to the pulling action of the gel. We cannot rule out a diminished affinity of the rigid RBC membrane for the gel as another possible cause for the reduced deformation by "pulling". Use of the patterned gel actuator revealed that the compression forces on the cells originating from the expansion of the gel below the LCST were high enough to deform the rigid RBCs, which ruptured because of their fragility (Figure 1G).

Polymeric actuators offer several advantages over single cell techniques such as optical tweezers or micropipettes. First and foremost is the simplicity by which such polymeric actuators can be prepared and applied. The versatility of PNIPAM chemistry allows adjustments of the LCST and the incorporation of biomolecules such as specific ligands, thus offering great flexibility in the design and use of such external force generators. A major advantage compared to single cell techniques is the simultaneous application of forces to a large number of cells or whole cultures (Figure 1H,I). This feature permits, for example, the study of mechanotransduction using standard biochemical techniques, which require substantial amounts of the investigated biomolecules, and the generation of data sets suitable for statistical analysis. Although one currently lacks the ability to apply and control forces of known magnitude, such as is possible with micropipettes or optical tweezers, we anticipate that standardized gels of known and tunable compliance at different temperatures will become available.

Supporting Information Available: Experimental preparations, as well as suggested applications. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA067171+