Scanning force microscopy of thin gelatin films on mica reveals two distinct film components with characteristic frictional, morphological, and adsorptive signatures. A high-friction continuous film 1–4 nm thick strongly adheres to mica, while a low-friction component is more weakly adsorbed as large islands on top of, or small domains within, the high-friction layer. The low-friction component exhibits a porous morphology and fluid-like character and is selectively destroyed when the film is heated sufficiently. A high-force scanning procedure remarkably transforms the molecularly-rough high-friction film into the molecularly-smooth low-friction component if a sufficient amount of water is present in or on the film. The nanostructure of both the high- and low-friction components is imaged using a nanometer-scale asperity tip. The anticipated network structure of gelatin is observed on the high-friction layer. The low-friction material is interpreted as moieties of gelatin attached to the molecularly-smooth low-friction component with a porous structure, resembling the anticipated network structure of gelatin.

1. Introduction

Scanning force microscopy (SFM) is emerging as a premier tool for characterizing organic thin films. The unprecedented capability to probe with a single instrument tribological and mechanical properties, long- and short-range surface forces, and even the chemical nature, as well as atomic- or molecular-scale surface structure, is manifest in recent SFM studies. A landmark achievement was the first resolution of chemically dissimilar surface regions by Meyer, Overney, and co-workers via differences in the frictional interaction with the SFM tip.

The major role of gelatin in the photographic, pharmaceutical, and food industries, as well as in holography, binding, and coating, attests to its general utility. Despite well-established product applications, gel research remains extremely active, primarily because of the complexity and richness of the subject. Gelatin is derived from collagen, the primary protein component of animal connective tissue, and comprises the ubiquitous binding agents which couple select functional groups. In our current research we are applying SFM to probe the mesoscopic- to molecular-scale structure and properties of short- and dry-swollen gelatin films, and their dependence on both intrinsic and extrinsic molecular coupling mechanisms.
Our attempts to maximize the resolution of structure in gelatin films led to the present study of thin films on a rigid hydrophilic substrate (mica). This architecture also allows us to determine layer thickness precisely and probe the strength of film adhesion by selectively removing portions of the film with high-force scanning. Our results reveal two film components with distinctly different frictional, morphological, and adsorptive character. A high-force scanning procedure remarkably transforms the high-friction, primary component into a low-friction material. Comparison of the properties of both types of low-friction moieties (as-deposited and scan-transformed from high-friction component) indicates that the materials are the same. We present molecular interpretations of these findings on the basis of protein folding, apparently induced by high-force scanning.

2. Experimental Details

Aqueous 10⁻³ wt % gelatin solution was prepared by slowly heating (~2 h) a 1 wt % mixture of gelatin (Kind and Knox photographic grade, type 2688) in distilled/deionized water (DW) to ~60 °C, followed by dilution with DW at ~20 °C. Freshly-cleaved muscovite mica (Union Mica Corp.) substrates were rinsed in DW, immersed in the 10⁻³ wt % aqueous gelatin solution (at least 2 h old initially) for 3 h, rinsed in a DW bath, carefully extracted to retain a residual puddle of water, covered, and allowed to dry slowly overnight in moderate-humidity (35% < RH < 60%) conditions. The films were initially imaged in air the following day. Repeated imaging over a period of several months reproduced the same qualitative film morphology. The Nanoscope III (Digital Instruments) SFM, a beam-deflection instrument, was used for all film characterization. Topographic and frictional force images were simultaneously collected at constant vertical cantilever deflection using triangular micro-fabricated 100μm cantilevers (spring constant = 0.58 N/m) with pyramidal Si₃N₄ tips. Some of these tips were coated with Cr at the manufacturer; in air these native chromium oxide tips were briefly used to compare measurements with those employing Si₃N₄ tips. All of the results reported here were obtained with the Si₃N₄ tips unless specified otherwise. Contact forces during imaging were in the 0.2–50 nN range. We attempted to minimize this force when possible; however, applying negative loads to offset tip-sample attractive forces often resulted in the loss of contact at surface asperities. The Si₃N₄ tip spring constant varied from 0.58 to 3.0 N/m, with vertical deflection ranges of 160-470 μm used. Images were collected with the tip scanning left-to-right in the sample's inertial frame; note that in the laboratory frame the tip remains fixed while the sample is correspondingly scanned right-to-left. Friction-actuated cantilever torsion was enabled by choosing a fast-scan direction perpendicular to the primary cantilever axis. Multiple imaging locations were systematically investigated on each sample to ensure reproducible and representative results.

Friction loop data were collected in the "y-disabled" mode, where scanning is performed along the fast (x), but not the slow (y), scanning axis. Region-specific relative frictional forces were measured by collecting a single topography/friction trace over a left-to-right/right-to-left scanning cycle containing all surface regions to be compared. Friction was quantified as the difference of lateral forces sensed upon scanning in opposite directions, averaged over all data points pertaining to a single surface region. Asperity-related contributions to cantilever torsion averaged to approximately zero over a complete cycle, leaving only the nonconservative dissipative term. The applied load was varied by changing the vertical cantilever deflection maintained during scanning. Unless specified otherwise, lateral scanning was performed at a frequency of 10 Hz; tip speed is then given by 2/scan length/10 Hz). Region-specific characterization of force versus distance was achieved in "force-displacement" measurements, i.e. vertical cantilever deflection as a function of vertical sample displacement toward and away from the chosen surface region. Tip-sample "adhesive" forces (including those due to capillary condensation) were quantified from the maximum cantilever deflection toward the sample during withdrawal.

Figure 1 contains representative topography (left) and frictional force (right) images of as-prepared gelatin film; higher elevation or frictional force is rendered brighter: (a) 20,000 x 20,000 nm region displaying scattered low-friction islands; (b) a typical magnified view (3000 x 3000 nm) of two islands with lower and upper surfaces approximately 1.5 and 6-10 nm above the continuous "first-layer" film; (c) a 2000 x 2000 nm region displaying low-friction domains ~100 nm across, and two high-friction surface elevations differing by ~0.5 nm.

3. Results

3.1. Gelatin Film Characteristics. In this section we present representative SFM images of gelatin film topography and tip-sample frictional forces. Surface regions differentiated in the friction images are further compared via force-displacement, as well as friction-load, measurements. Removal of film layers with high-force scanning aids in quantifying layer thickness and adhesion. Figure 1 contains representative topography (left) and friction (right) images of as-prepared gelatin films collected at contact forces of several tens of nanonewtons. Higher elevation or frictional force is rendered brighter. Figure 1a surveys a 20,000 x 20,000 nm region revealing scattered islands ~500–5000 nm in lateral extent, primarily 1.5 nm in height, and exerting a reduced frictional force on the SFM tip relative to the surrounding surface. Island number density is of the order 10⁶ cm⁻². The scan rate was reduced to 3 Hz for such large-scale images. A magnified view of two islands is shown in the 3000 x 3000 nm image of Figure 1b. All surface regions are rough on the atomic scale, indicating that the mica surface is completely

covered with gelatin, at least to the extent that the SFM tip is constrained from reaching the substrate. The lowest regions, which we will call the first layer, appear "grainy" as imaged, the smallest resolved "grains" being several tens of nanometers in lateral dimension. A relatively large frictional force is exerted on the SFM tip in these regions. Typical modulations in surface elevation on the first layer are about ±0.5 nm. The mean elevation is reduced by approximately 0.5 nm in a region roughly 100 nm wide surrounding the islands. This is difficult to perceive in Figure 1a and 1b but was repeatedly quantified in detailed analysis of scan profiles. The islands contain subregions of characteristically different thicknesses. The dominant, thinner island portions have mean elevations 1.5 nm higher than those of the first layer and the same elevation variability (±0.5 nm); however, they contain circular pores 10–100 nm in diameter and exhibit lesser friction. Friction identical to that of the first layer is imaged at the bases of the pores. The thicker island regions have variable elevation 6–10 nm above the first layer and a friction signal identical to that of the thinner island regions.

The image in Figure 1c (2000 × 2000 nm) displays additional features observed in the thinnest films studied (quantified below), namely a "first layer" containing regions of two distinct mean elevations differing by ±0.5 nm and small domains (<100 nm across) with a friction signal identical to that of larger islands like those in Figure 1b. No substructure was imaged in the topography or friction of these domains, e.g., no granular morphology.

Friction–load measurements quantified the region-specific frictional differences seen in Figure 1. At this juncture we compare only the relative frictional forces on different surface regions; we will later present absolute frictional force data for all sample regions. Figure 2a contains representative friction versus load data on both the first layer (open circles) and a 1.5 nm thick island (closed circles). Linear fits of first layer and island data at applied loads below ≈20 nN are shown as solid and dashed lines, respectively. In this load range the frictional force \( F_f \) clearly obeys Amontons’ law \( F_f = \mu F_N \), where \( F_N \) is the contact force (applied plus adhesive loads) and \( \mu \) is the coefficient of friction. Above an applied load of ≈20 nN the frictional force on the island departs from the linear trend, rising more substantially with increasing load, while the first-layer data remain consistent with the initial trend. Departure from linearity on the first layer occurs above an applied load of 100 nN.

Figure 2b compares typical force–displacement data taken during approach toward (thin lines) and withdrawal from (thick lines) first-layer (solid lines) and island regions (dashed lines). The sample displacement scale is zeroed at the position of greatest approach during the measurements.

The departure of the friction–load relationships from initially linear trends as a function of increasing load, such as displayed in Figure 2a, generally coincided with the onset of permanent deformation (wear) observed in topography images. This led to attempts to remove film layers to quantify thickness and adhesion, usually performed in the \( y \)-disabled mode. The image monitored during continuous \( x \)-scanning was an evolving plot of one-dimensional topography/friction versus time. When changes were no longer observed, the scan line was displaced incrementally along \( y \), the process repeated, scan displaced again, etc. After several repetitions, the affected strip was of order 100 nm wide (\( y \)) times the length of the scan in \( x \). The result of such a process applied to the film region of Figure 1b is presented in the topography/friction images (left/2) of Figure 3 (also 3000 × 3000 nm), collected at a contact force of 100 nN yields the quick removal of the thinner island from the 3000 nm scanned strip but left the first layer intact. The result is visible near the bottom of Figure 3.
process as the removal of the low-friction material to yield the underlying first layer, which apparently covers the entire mica surface. No intermediate elevation or frictional force was observed while this process was monitored; i.e., no evidence for vertical island substructure (e.g., layering) was obtained. No "piling up" of the removed material was observed in the surrounding regions. Analogous results were obtained on the thicker island regions, but at lower forces; the thick island at the top of Figure 1b was quickly removed from the scanned strip at a contact force of only 50 nN, leaving intact both the first layer and thinner island portions.

At contact forces of \( \approx 150 \) nN the first layer can be removed (section 2), yielding cleared regions of an atomically-flat surface; surface structure with the hexagonal atomic periodicity of mica was imaged at these locations. Film thickness was determined by measuring film surface elevation relative to the cleared mica surface. We measured first-layer mean thicknesses of 1–4 nm among all the films thus characterized (\( \approx 20 \)), uniform to within \( \pm 0.5 \) nm for a given film.

The onset of tip-induced modifications was not strictly a function of force: scan-derived changes took place on the low-friction component at very slow scanning speeds, applying what were otherwise nonperturbative forces. Removal of small low-friction domains like those in Figure 1c was accomplished by repeatedly raster scanning a small region \(( < 100 \times 100 \text{ nm} )\) containing only these domains at speeds 2 orders of magnitude slower than those used to collect that image. Continuous imaging eventually yielded an atomically-flat surface with the hexagonal atomic periodicity of mica. No intermediate elevation or frictional force was imaged during the entire exercise. Subsequent larger scale images revealed a hole in the gelatin film in place of the original low-friction domain. Comparison of images collected before and immediately after this treatment indicated a domain thickness of 1.5 nm. Remarkably, the removed domains gradually reappeared in both topographic and frictional force images over a time scale of minutes.

We have begun extensive studies of the dependence of friction on scanning speed. We preliminarily report that the same region specificity of frictional force was observed independent of tip speed throughout the range examined \((0.2–500 \text{ \mu m/s})\). At tip speeds on the order of 1 \( \text{\mu m/s} \) or less we generally observe an increased amplitude of variations in frictional force during a single scan, relative to measurements at higher speeds. These variations become particularly large at speeds below \( \approx 0.5 \text{ \mu m/s} \) and are not accompanied by similar modulations in the vertical cantilever deflection (we disabled the feedback circuit to rule out feedback-induced oscillations as the source of measured frictional variations). Thus at these low speeds the friction has a more broken, nonuniform character.

3.2. Transformation from High- to Low-Friction Components. In this section we present representative topography/frictional force images of gelatin films modified by raster scanning square regions 500–2000 nm wide at contact forces of \( \approx 150 \) nN. Subsequent, time-dependent morphological changes are imaged. The stability of the transformed films with respect to tip penetration and disruption is investigated, and friction–load measurements are compared between surface types.

Figure 4 contains topography/frictional force images (left/right) of a 2000 x 2000 nm sample region illustrating the time evolution of a 1000 x 1000 nm region which previously had been scanned at a contact force of \( \approx 150 \) nN to remove the high-friction first-layer gelatin. Elapsed times after termination of the high-force scanning process are approximately (a) 1, (b) 2.5, and (c) 6 min. The resulting low-friction film is 1.5 nm thick; the lower regions between film domains are the mica surface.

Figure 4 presumably contains removed gelatin. They

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(31) Exposed substrate regions imaged with the atomic periodicity of mica lack any evidence for the removal of a mica "flake". The presence of such a flake attached to the SFM tip has been postulated (see for example: Ovemey, G.; Tománek, D.; Zhong, W.; Sun, Z.; Miyazaki, H.; Mahanty, S. D.; Güntherodt, H. J. Phys.: Condens. Matter 1992, 4, 4233) as the source of imaged contrast patterns with atomic periodicity on layered materials (mica, graphite) at loads otherwise expected to produce damage, i.e. at which the force per unit area exerted by a truly atomic-scale tip would exceed the yield strength of the material (as it would in the present work). Our results suggest a different imaging mechanism.
display the highest friction of all surface regions and are easily perturbed by the SFM tip, accounting for the changes in mound shape in Figure 4. Scanning at smaller forces generally prevented this, but the time-evolution of transformed films was nonetheless observed, indicating no correlation with changes in the border material induced by imaging. Invoking careful procedures, we generally found that the film growth was intrinsically time-dependent, i.e. not induced by SFM scanning. (For example, after a first image was collected, we moved the tip far from the affected region for >30 min and then returned to image a steady-state film much larger than initially imaged.)

All such transformed films imaged (>80), whether produced in regions 500, 1000, or 2000 nm on edge, were 1.5 ± 0.2 nm thick (variance within a particular film was <0.1 nm) and exhibited friction matching (to within the noise) the low-friction islands/domains present on the original film, irrespective of the first-layer thickness (1–4 nm). In some cases the steady-state transformed film completely covered the modified region, even if the original film was only 1 nm thick (as in Figure 4), apparently signifying a net gain in film volume. The possibility that water comprised the additional volume led us to investigate the importance of ambient humidity on the transformation phenomenon. We discovered preclusion by dry ambient conditions (RH ≤ 30%): the low-friction film was repeatedly absent when transformation was attempted in 500 × 500 nm regions, leaving instead a permanently bare mica surface. After the sample was exposed to saturation water vapor conditions briefly (<1 min; followed by ambient drying), subsequent high-force scanning yielded extensive low-friction films in every attempt. The entire experiment was repeated with the same sample after 24 h in dry ambient conditions (expected to dry the film29), yielding the same result: no low-friction material resulted from high-force scanning before exposure to high humidity, while ambundant quantities resulted from the same procedure immediately after the exposure. The same behavior was confirmed with identical experimental procedures on other samples which had remained in dry conditions.

Immediately after the transformation process, films like that in Figure 4 were susceptible to penetration by the SFM tip (the susceptibility being a function of the state of the tip, section 3.3): while initial imaging attempts were monitored, it was often observed that the SFM tip was penetrating the film (described below) and thereby inducing the formation of small pores 10–100 nm in diameter within the film domains. This was best avoided by substantially reducing the contact force (applying a negative load) and avoiding the steep gelatin mounds framing the modified region (to preserve tip-sample contact).

Typical results illustrating the penetration phenomenon are presented in Figure 5, which shows 1500 × 1500 nm topography/friction images (left/right) of a film transformed at a contact force of ≈150 nN. Figure 5a was captured after the contact force had been lowered to ≈6 nN; the transformed-film thickness is 1.5 nm, with friction identical to the low-friction islands/domains in the original film. Regions between the film domains are the mica surface; the film–mica friction contrast has been enhanced relative to that of Figure 4. SFM tip penetration was subsequently investigated by increasing the contact force slightly, collecting an image, increasing the force again, collecting an image, etc. Two of these images are shown in Figure 5b and 5c, collected at contact forces of ≈14 and ≈25 nN, respectively. Partial (Figure 5b) and total (Figure 5c) penetration by the SFM tip is indicated by the tendency toward zero differentiation in topography, reflecting contact with the mica. Consistent with this interpretation is inversion of the film–mica friction contrast seen in both images, which we attribute to greater resistance as the SFM tip plows through the film domains. (The frictional forces cannot be compared quantitatively between the different images because the midpoint of the friction contrast scale in each is freely shifted to the image average). After the image of Figure 5c was collected, the contact force was again reduced to ≈6 nN and an image collected, as shown in Figure 5d. Remarkably, the overall
shape of the film domains was largely unperturbed by the SFM tip penetration. The domains continued to grow laterally during this exercise while a 1.5 nm thickness was maintained. Tip penetration apparently yielded pores larger in diameter but smaller in number, within the individual domains (compare Figure 5d to 5a).

Once stabilized, the transformed films could be imaged at "moderate" contact forces, the magnitude of which was a function of the state of the tip (section 3.3). However, repeated moderate-force raster scanning at very slow speeds was observed to remove the film gradually (leaving the mica surface), mirroring the removal of the low-friction domains within the initial first layer (reported in section 3.1).

Figure 6 (3000 × 3000 nm, contact force ≈ 20 nN) contains five surface regions exhibiting characteristic friction. A 2000 × 2000 nm region of first-layer gelatin had been scanned briefly at a contact force of ≈ 150 nN to transform the film partially into low-friction material. The lowest elevation in the modified square region corresponds to the mica surface, and the highest regions contain first-layer gelatin which has been "disrupted" by high-force scanning but not yet transformed into the low-friction component. Outside of the modified region lies the unperturbed first-layer gelatin and small low-friction islands. The trend for frictional force is $F_{\text{disrupted}} > F_{\text{first layer}} > F_{\text{mica}} > F_{\text{island transformed}}$, observed in all cases examined independent of tip, humidity, and film age.

After regions like that in Figure 6 were produced, we measured frictional force versus load on multiple surface types simultaneously. Representative results at applied loads producing no wear are presented in Figure 7, for the surfaces of first-layer gelatin (solid squares), a low-friction island (solid diamonds), a transformed film (open diamonds), and bare mica (solid triangles). A linear fit of each data set is shown (solid lines), the slope of which quantifies the frictional coefficient. The absolute frictional force calibration was obtained with a method described in the Appendix.

An understanding of the contribution of tip condition, film water content, scanning speed, etc. to this variance is the subject of ongoing work.

We have not extracted a definitive coefficient of friction on scan-disrupted regions because of substantial variability among the cases studied and difficulties with sporadic further plastic deformation and clinging of gelatin to the tip. In general, the frictional force on these regions during stable scanning was more than double that on unmodified first-layer gelatin.

3.3. Film Nanotechnology. We have found that during force-displacement measurements, by the repeated ramping of $Z$ to yield contact forces of at least several hundred nanonewtons, the tip-sample adhesive force decreases gradually over a period of 2–3 min from several tens of nanonewtons to as small as ≈ 2 nN. A tear in the gelatin film extending to the mica substrate results at the site of this procedure. Subsequent force-displacement measurements at unperturbed locations (and on different samples) reveal an identically small adhesive force, indicating a change of tip state. Figure 8 compares representative force-displacement data collected during withdrawal from unperturbed gelatin initially (line) and after the above Z-ramping procedure (solid circles); the maximum attractive forces are ≈ 40 and ≈ 2.5 nN, respectively. Approach data were subtracted from the raw withdrawal data to remove shallow, long-range oscillations due to optical instrumental effects. The sample displacement scale is zeroed at the jump-to-contact location; the initial withdrawal data (line) are off scale at $Z = 25–65$ nm. With the initial tip, the loss of contact during withdrawal occurred suddenly, when the cantilever spring force exceeded the attractive tip-sample interaction. For measurements in ambient conditions (as in our case) the required force presumably contains a large contribution

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(32) If the initial adhesive force is more than ≈ 150 nN, a reduction does not take place at the highest force attainable (≈ 1.5 μN). In this case the implicitly large contact area may limit the apparent too small to exceed the "activation barrier" for contaminant removal or gelatin deposition. Note that an approximately 15° cantilever incline results in slight lateral tip displacement in the contact regime during force-displacement measurements; this is clearly evident in linear gouges lying in the XY plane produced by ramping to very high forces. The critical quantity for tip modification therefore may be a large shear stress.

due to capillary interactions of water (contained in or on the gelatin film\textsuperscript{30}) and possibly interfacial contaminants.\textsuperscript{34}

Following the above treatment, the attractive forces fall off rapidly but continuously for the first 5 nm beyond the point of maximum attraction, and then very slowly for another $\approx$150 nm. A sudden cantilever jump away from the sample was only observed in cases where the gradient of attractive force exceeded the cantilever spring constant. The adhesive force for the modified tips often exhibits a strong dependence on $Z$-ramping treatment; a decrease of $3$ orders of magnitude in rate can result in an increase of tip-sample adhesion by more than a factor of $5$. No such rate dependence has been observed with tips in the initial state.

Images collected with the modified tips display dramatically-enhanced resolution. This is illustrated in the representative images of Figure 9, each collected at contact forces of $<1$ nN; low-pass Fourier filtering removed most of the small-wavelength noise comprising the streakiness normally visible along the fast-scan axis. Figure 9 contains a topographic image of a 300 x 300 nm region on first-layer gelatin prior to the $Z$-ramping procedure; a large negative load ($\approx -20$ nN) was applied to minimize the contact force. A granular morphology was imaged, the smallest "grains" being about 40 nm in diameter. The total measured range of surface elevation is 2.1 nm. Figure 9b shows a same-sized region of the same film imaged following the great reduction of the adhesive force; only a small negative applied load ($\approx -2$ nN) was needed to minimize the contact force. Here the imaged morphology appears fibrous (characteristic of gelatin\textsuperscript{29,34,35}), with measured fiber width less than 10 nm. An amorphous network of fiber segments is revealed with typical segment lengths $\approx 20-30$ nm. The total measured range of surface elevation is 3.0 nm. Successive images collected on a 1000 x 1000 nm region contained an identical, detailed network of fiber segments. A similar fibrous morphology was imaged on the recessed regions surrounding islands like that in Figure 1b, and on the 1.5-nm thick island surfaces.

The high-resolution imaging capability of the above-modified tips is accompanied by a greater propensity for film disruption. Plastic deformation occurs in general at substantially lower contact forces; at a contact force of $\approx 150$ nN, complete removal of first-layer gelatin from a

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Figure 11. Topography/frictional force images (left/right) of different surface regions of the same gelatin film at room temperature after being heated to (a) 37 °C for 40 min and (b) 51 °C for 20 min. The islands in part a are 1.5 nm in height.

4. Discussion

4.1. Resolving Network Structure. The conventional picture of a gelatin gel is a three-dimensional network of conformationally-free polypeptide strands 0.5 nm in diameter, with rod-like triple-helical (collagen fold) cross-links 1.5 nm in diameter.23 Electron microscopy images of a gelatin gel following metal coating revealed randomly oriented linear filaments which were interpreted as triple-helical strands.23 Nonperturbative imaging of the gelatin network structure has not been reported (to our knowledge) prior to the present study. Our initial attempts to do so with conventional SFM tips apparently were limited by the large tip-sample contact area which results when tip-sample attraction yields substantial compliance in a soft sample. Imaging in water in hopes of minimizing attractive capillary forces only worsened the problem, because of the much softer water-swollen state of a gelatin network23,24 (we will report SFM investigations of this important state in a forthcoming article).

We propose that the Z ramping procedure which dramatically reduces the tip-sample adhesive force (Figure 8) and enhances imaging resolution (Figure 9b) creates a gelatin “nanoasperity” on the end of the SFM tip.36 An increasing tip-sample adhesive force with decreasing Z scan rate then presumably reflects enhanced gelatin–gelatin interdigitation for longer times spent in contact.37 Interdigitation in principal should yield measurable “clinging” of gelatin molecules, sensed as attractive forces, up to distances on the order of 1 molecular length (300 nm); this was confirmed experimentally (e.g. Figure 8). Because gelatin and Si3N4 are both hydrophilic, we have no reason to expect a much stronger Si3N4–gelatin interaction than gelatin–gelatin interaction (especially in air with presumably dominant capillary forces); hence the large drop in adhesive force is apparently due to a much smaller contact area. Imaging resolution and propensity for damage increased gradually and monotonically as a function of decreasing adhesive force, expected for a diminishing contact area.

Applying a negative load to minimize the contact force improved resolution by roughly a factor of 2 compared to that at zero applied load, independent of the magnitude of adhesive force. These observations suggest compliance with the JKR theory of adhesive contact between curved surfaces,38 including the effects of capillary condensation.39 Within this theoretical framework, tips maintaining a very small contact area apparently have a sharply reduced radius of curvature. Experimental results support this: the ability to distinguish the mica surface in transformed-film pores as small as 10 nm in diameter (Figure 10) with a high-resolution tip implies a radius of curvature of less than 10 nm (from geometric considerations), much smaller than generally known for unmodified pyramidal SFM tips. In another article,39 we will present an exhaustive analysis of force-displacement measurements and electron microscopy images to better elucidate the nature of the gelatin “nanotips”.

The fiber width measured in SFM images (several nanometers in Figure 9b) is a convolution of the true fiber diameter and the shape of the imaging asperity.39 The molecular spacing in thin gelatin films is apparently sufficient to allow our “sharp” gelatin tips to map out individual molecular-scale fibers but is not sufficient to allow the bare Si3N4 tip to do the same, resulting instead in a granular image with reduced surface “corrugation”. The variable elevation (brightness) of the strands in Figure 9b indicates three-dimensional network character even in a gelatin film only 3.3 ± 0.5 nm thick. Gelatin networks in the thinnest films imaged (<1 nm) displayed reduced variability in elevation, indicating more two-dimensional character. Images of micrometer-scale films (prepared from 1–10 wt % gelatin solutions in a parallel study) reveal greater elevation variability than any of the films discussed here, presumably reflecting a “bulk” network conformation.

High-resolution images like those in Figure 9b and 10 demonstrate the utility of our method of constructing ultrasharp SFM tips. However, these gelatin tips have limited resilience: observations indicate they eventually break off or become blunt. The former is suggested by a...
sudden return to the characteristic, rate-independent adhesive force of the bare tip (Figure 8). The latter is reflected in a rate-dependent adhesive force much greater, and imaging resolution much worse, than that of the original bare tip. Often this cannot be rectified by further scanning procedures; however, tip immersion in hot tap water (60 °C) for ≤1.5 min, followed by its dipping in water rinse, subsequently yields the characteristic bare-tip adhesive force and the ability to construct a sharp gelatin tip.

Blunt gelatin tips only weakly perturb the first-layer gelatin upon raster scanning at ≈150 nN, indicating that a critical parameter for film modification is in fact force per unit area. This concept is consistent with careful observations of the first-layer morphology during modification with a sharp tip: at any instant the effect appears to be localized to the tip-sample contact region, i.e. not the result of nonlocal heating. Consequently, the region modified during high-force raster scanning is only the scanned region. This is in contrast with effects of high-force scanning we have observed occasionally on AgBr surfaces, where a square trench corresponding to the scanned region was accompanied by a larger circular “halo” of growth in the film, apparently a region heated sufficiently to disrupt material cohesion.

4.2. Low-Friction Moieties. Low-friction islands/domains observed in as-prepared gelatin films (Figure 1), and low-friction films resulting from the transformation of first-layer gelatin by high-force raster scanning (Figures 4–6,10), display numerous identical characteristics: (1) thickness (primary island elevation), (2) coefficient of friction, (3) reduced adhesion with tip relative to first-layer gelatin, (4) larger jump-to-contact relative to first layer, (5) circular pores primarily 10–100 nm in diameter, (6) fluid-like conformity to the structure of the underlying surface (mica or first-layer gelatin), (7) easy removal from the mica surface at slow scanning speeds, and (8) time-resolved growth on mica. These striking similarities lead to the conclusion that the materials are the same. We propose that the low-friction moieties contain intramolecularly-folded, triple-helical gelatin (diameter = 1.5 nm). Such folding is understood to take place in low-concentration aqueous gelatin solutions such as that employed in our film preparation (we have not observed low-friction moieties in films prepared from 1–10 wt% solutions).

Our results imply that intramolecular folding can be induced in thin gelatin films on mica by high-force SFM scanning when an adequate amount of water is present in or on the film. The collagen-fold triple helix contains hydrogen bonding between CO and NH groups, both directly and via interstitial (structural) water molecules. The net volume gain we observed for some of the transformed gelatin films suggests that some of the “free” water initially present in the vicinity but not “locked” in the film structurally (and thus not contributing to its measured thickness) is converted to structural water in the intramolecularly-folded moiety. The time-resolved growth of the film presumably occurs as free gelatin molecules (whose transience and lack of rigidity render them invisible to topographic imaging) fold intramolecularly, incorporating free water molecules as needed at interstitial sites within the triple helix. Fluid-like character in the folded moieties is not inconsistent with high “bound” water content, because such water exhibits mobility intermediate between solid and liquid water, i.e. is not truly “locked” in place.

Heating the as-prepared film to 37 °C probably drives enough water out of the film to substantially reduce the size of the folded moieties (Figure 11a), while at 51 °C all folding is destroyed (Figure 11b) as a result of this dehydration. Similarly, the observed variance in the lateral size of different transformed films at steady state probably reflects the quantity of free water available in/ on the initial film, in turn a function of the ambient relative humidity during the period of investigation. (Nominally-dry gelatin films are understood to contain 10–20% water by weight at relative humidities in the 20–60% range.) Under especially dry conditions (RH < 30%) where no low-friction material was produced in a 500 × 500 nm region, we repeatedly obtained significant amounts in 2000 × 2000 nm scans. Apparently, water not only comprises a certain fraction of transformed-film volume but also mediates the folding process (as expected): increasing the size of the scanned region allowed the accessed water to attain local concentrations high enough to mediate folding. Further experiments under controlled humidity should produce a more precise understanding of the role of water.

The jump-to-contact is often preceded by negligible cantilever deflection (as seen in Figure 2b) and apparently is not initiated by a cantilever-related instability (i.e. attractive force gradient exceeding cantilever spring constant); the event appears to be initiated instead by an interfacial (sample/tip) jump which is larger on the folded moieties, consistent with a less stiff material. Variations in mechanical compliance reflecting the range of contact forces among images (0.2–50 nN) may account for the variability of the measured thickness (∼0.2 nm), given a presumably soft, high-water-content moiety. At very stable contact forces of ≈0.2 nN (with gelatin nanotips), the measured transformed-film thickness was ∼0.2 nm greater than the mean value of 1.5 nm, suggesting the equivalent of one additional layer of water in the film or between tip and film when the contact force is very small.

The easy removal of the folded moieties by applying moderate forces implies weaker adhesion to mica or first-layer gelatin than the first-layer/mica adhesion, consistent with the dominant order of adsorption. (Determination of the absolute adhesion energy from the force applied is problematic because of the scan-rate dependence in the force required for removal, i.e. the implicit lack of thermodynamic equilibrium.) On mica the intramolecularly-folded gelatin molecules apparently lie precisely flat, yielding a uniform 1.5 nm thick layer, while the molecular strands in the original high-friction conformation do not, as indicated by the greater variability of surface elevation in Figure 9b compared to Figure 10. This distinct difference may reflect the expected residence of hydrophobic groups in the interior of a folded protein (shielded from the aqueous medium), leaving primarily hydrophilic groups to interact more uniformly with the hydrophilic mica substrate. Our results indicate reduced overall film adhesion in the folded case, however, suggesting that a significant number of functional groups (e.g. those engaged in hydrogen bonding within the triple helix) which would otherwise interact strongly with mica are precluded from doing so in the folded case.

4.3. Friction Mechanisms. Although the field of tribology has existed for decades, progress has been made only recently in probing the fundamental mechanisms of friction on a molecular to atomic scale with sensitive instruments, i.e. SFM and variants of the surface

References

forces apparatus.\textsuperscript{37,47} The molecular mechanisms underlying frictional processes involve the same nonequilibrium dynamics manifest in the hysteretic measurements of surface forces (approach vs withdrawal) deformation (loading vs unloading), and contact angle (advancing vs receding)\textsuperscript{37,47}. This hysteretic behavior has been categorized by Israelachvili as either "mechanical" or "chemical" in nature.\textsuperscript{37} Mechanical hysteresis is reflected in "stick-slip" processes involved in friction. Nonequilibrium conditions during slip occurs in two types of interfacial systems: (1) between solid surfaces in contact when the tangential stress gradient exceeds the effective elastic spring constant for elastic shear deformation,\textsuperscript{46,48} and (2) on a confined lubricant layer when the shear stress exceeds a static yield point (such layers display quasisolid properties when confined to molecular-scale dimensions).\textsuperscript{48} Chemical hysteresis occurs when the configuration of molecules changes. This is certainly the case when plastic deformation occurs; it also applies when an equivalent configuration ultimately results but only after molecular relaxation has dissipated a portion of the deformation energy (i.e. viscoelastic deformation). In the present work elevated friction reflecting plastic deformation was verified during all tip-tipped modulation and deformation, when at least one material is a polymer, necessarily involves viscoelastic behavior,\textsuperscript{48} which derives from long molecular relaxation times. Energy imparted to the polymer by shear and tensile stresses is only partially stored as elastic energy, which by definition is reversibly transferred. The remainder of the transferred energy is ultimately dissipated via molecular relaxation. It is well known that viscoelastic dissipation in polymers, though occurring predominantly in the interior of the material, contributes strongly to interfacial friction; in general it is difficult to uncouple surface vs "bulk" viscoelastic effects.\textsuperscript{55,56}

The viscoelastic nature of water-swollen gelatin networks has been thoroughly documented.\textsuperscript{32,44} The same dissipative physics in nominally-dry networks (as presently studied) could friction relative to an elastic material like mica. Similarly, we reported elevated friction on gelatin relative to graphite.\textsuperscript{4} Gelatin molecules should have greatly-reduced relaxational freedom when contained in the rigid, triple-helical tertiary structure, whether as members of an intermolecular cross-link in a network or as intramolecularly-folded entities. In the present study we hypothesize that the disrupted film regions display the highest friction because of a sharp reduction in the number of triple-helical cross-links,\textsuperscript{31,24} caused by high-force scanning. The folded moieties exhibit the lowest friction because they contain gelatin molecules primarily, or perhaps exclusively, in the triple-helical conformation. The fluid-like nature of the folded moieties additionally suggests that shear deformations are not

\textsuperscript{(1)} Germann, G. J.; Cohen, S. R.; Neubauer, G.; McClelland, G. M. J. Appl. Phys. 1993, 73, 163.


\textsuperscript{(46)} The presence of boundary lubricants can reduce or eliminate solid-solid adhesion and diminish the shear forces acting on the surfaces, and may yield a linear friction-load relationship.\textsuperscript{59,61} Comparative measurements of adhesive forces, $F_{ad}$, do not indicate substantial differences in contaminants\textsuperscript{34}(potential lubrication) on the various surfaces investigated in this study. After it is normalized to the estimated tip radius of curvature $R$, we find that $F_{ad}/R$ is of the order 1000 mJ/m$^2$, much larger than expected values.\textsuperscript{37,59,53} This presumably reflects a large contribution due to the capillary forces of water.\textsuperscript{34,37} On molecularly-smooth surfaces like mica or the low-friction gelatin moieties on mica, water confined to molecular-scale dimensions between sample and tip could order into one or more solid-like molecular layers\textsuperscript{37,53} so that the tip might ride atop a "boundary lubricant" layer of water. Our current concern is whether such an effect (if present) might vary substantially among the surface types investigated. The formation of distinct layers of confined water should be precluded on the molecularly-rough\textsuperscript{49} (a) first-layer network, (b) low-friction islands (whose surface texture mirrors the underlying first layer), and (c) scans of disrupted gelatin (highest friction region). Measured friction on the molecularly-rough low-friction islands is identical to that on the molecularly-smooth transformed films, which ostensibly would be more conducive to boundary-water layering. We conclude that the observed material-specific friction does not derive from differences in lubrication by a confined boundary layer of water.

The chemical hysteresis of intersurface adhesion or deformation, when at least one material is a polymer, necessarily involves viscoelastic behavior,\textsuperscript{55,56} which derives from long molecular relaxation times. Energy imparted to the polymer by shear and tensile stresses is only partially stored as elastic energy, which by definition is reversibly transferred. The remainder of the transferred energy is ultimately dissipated via molecular relaxation. It is well known that viscoelastic dissipation in polymers, though occurring predominantly in the interior of the material, contributes strongly to interfacial friction; in general it is difficult to uncouple surface vs "bulk" viscoelastic effects.\textsuperscript{55,56}

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higher friction on mica may reflect the easy dissipation of energy as phonons in the crystalline, rigid material. Although the triple-helical intramolecular conformation is an ordered structure, and often termed “crystalline”, high-resolution images (e.g. Figure 10) display no long-range order in the folded moieties; thus phonon generation should be strongly impeded.

5. Summary/Outlook

Two distinct components in thin gelatin films adsorbed from aqueous solution onto mica were comparatively characterized with respect to morphology, friction, adhesion, and stability:

(1) A continuous “first layer” of variable thickness (1-4 nm) which exhibits a frictional coefficient more than twice as large as that on mica and which strongly adheres to mica.

(2) A film component primarily 1.5 ± 0.2 nm thick which exhibits a frictional coefficient roughly half of that on mica and adheres to the underlying surface more weakly than the first layer; this component was manifest as (a) porous islands up to several micrometers in lateral dimension located on top of the first layer, (b) small domains less than 100 nm across located within the first layer, in contact with the mica, and (c) films in contact with the mica after “transformation” from the high-friction first layer during a high-force raster scanning procedure.

Film nanostructure was resolved using a “nanoasperity” of gelatin; this became attached to the SFM tip during a Z ramping procedure which reaches contact forces of several hundred nanonewtons. The first layer exhibits the expected fibrous network structure of a nominally-dry gelatin gel. The low-friction component is interpreted as moieties of intramolecularly-folded, triple-helical (diameter = 1.5 nm) gelatin which presumably contain substantial structural water. High water content in this component was apparently manifest in its (1) precluded formation in dry ambient conditions, (b) fluid-like conformity to the surface topography of the underlying material, (c) time-elapsed morphological changes following transformation or modification, (d) enhanced jump-to-contact behavior, and (e) dehydration-induced destruction.

High-force scanning of the first layer presumably destroys physical cross-links and initially yields a third material exhibiting much higher friction, prior to formation of the low-friction component. Dissipative mechanisms were discussed to explain the frictional behavior on the three materials. We hypothesized that the magnitude of friction decreases with an increased amount of triple-helical conformation among the gelatin molecules, whether as members of intermolecular cross-links in a network or as intramolecularly-folded entities.

Many phenomena and procedures reported in this work are novel, with respect to SFM research in particular or materials research in general. These include the following: (1) spatially-resolved phases of a biopolymer, each with distinct morphological, tribological, and adsorptive character; (2) spatially-select perturbation/removal of specific film components to characterize thickness, adhesion, and stability; (3) locally-selected, force-activated phase transitions; (4) the sensitivity of interfacial protein folding to local water content; and (5) contact force imaging with a polymer nanoasperity. Our understanding of each of the above is certainly not complete and is being augmented with ongoing investigations of the rate dependence of friction, the role of tip state and adsorbed/
absorbed water in friction measurements, "ungelled" gelatin films, extrinsic (covalent) cross-linking, and water-swollen gelatin. Incorporating the methodologies reported in the present study makes the following fundamental or applied research possible: (1) local probing of dissimilar phases or adsorption states in polymer films, (2) SFM characterization of competing or multilayer adsorption processes, (3) probing molecular conformation locally via frictional dissipation, (4) novel phase transitions induced with scanning force methods, (5) local characterization of the nebulous protein folding process, (6) gelatin-film templates for selective adsorption by construction of scan-patterned, two-phase gelatin substrates, (7) gelatin films as (high spatial resolution) water vapor sensors, (8) scan-patterned gelatin-film optical devices, exploiting the modified optical activity of triple-helical gelatin.

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Appendix

Typical one-dimensional topography $Z(X)$ (solid circles, top panel) and friction loop (open circles, middle panel) data on transformed film domains on mica are shown in Figure 12a. The domain edge crossed by the vertical line lies perpendicular to the scanning direction (i.e. along the y-axis). The difference in left-to-right and right-to-left frictional forces is plotted as open squares (middle panel) containing the nonconservative component of the overall lateral force sensed. In the bottom panel, the sum of left-to-right and right-to-left frictional forces (open circles), the conservative component, is scaled appropriately and overlaid with the first derivative of the topography (closed circles). An enhanced (reduced) signal at the leading (trailing) edges of the domains presumably reflects additional cantilever torsion due to an off-vertical component of the tip-sample contact force. In earlier work we derived an equation relating cantilever torsion to the coefficient of friction and slope of topography ($dZ/dX$) along the scan direction, employing a spherical-tip model. Here we present an analogous derivation for a tip of radius $R$ and height $L + R$ scanning left-to-right across a discontinuous decrease in surface elevation (step) of height $h$, illustrated in Figure 12b. All surfaces investigated in the present work exhibited linear friction—load relationships (Figure 7) consistent with Amontons' Law $F_t = \mu F_n$, where $F_t$ is the tangential frictional force and $F_n$ is the contact force normal to the tip, provided that wear did not result in the load regime examined (recall the discussion of Figure 2a).

For a spherical tip sliding down the step in Figure 12b and at a height $Z$ above the lower surface, the torque $\tau_t$ about the principal cantilever axis due to the tangential frictional force $F_t$ is given by

$$\tau_t = F_t(L + R + Z) \cos \Theta = \mu F(L + R + Z) \approx \mu F_L$$

employing $F_t = \mu F_n / \cos \Theta$, where $F_t$ is the vertical component of the tip-sample contact force (maintained approximately constant via instrumental feedback), and the geometric approximations $R/L \approx 0.01$ and $Z/L \approx 0.001$ which apply to our system. The torque due to the normal component of the tip-sample contact force is

$$\tau_n = -F_t L \sin \Theta = -F_t L \tan \Theta$$

so that the total torque is given by

$$\tau = \tau_t + \tau_n \approx F_t L (\mu - \tan \Theta)$$  \hspace{1cm} (1)$$

We may alternatively express this in terms of the independent variable $X$. The profile mapped out by the tip in traversing the step is

$$Z(X) = (R^2 - X^2)^{1/2} + h - R$$

The measured slope is given by

$$\frac{dZ}{dX} = -\frac{X}{(R^2 - X^2)^{1/2}} = -\tan \Theta$$

Thus eq 1 can be expressed in terms of the slope of the measured topography,

$$\tau \approx F_t L (\mu + \frac{dZ}{dX})$$  \hspace{1cm} (2)$$

which is identical to the expression derived in our earlier work where $dZ/dX$ was instead the actual slope of the surface. This expression separates the total cantilever torsion into terms proportional to $\mu$ and $dZ/dX$, derived from nonconservative and conservative forces, respectively. In Figure 12a the excellent agreement of $dZ/dX$ with the conservative part of the measured cantilever torsion supports the validity of this expression. Importantly, eq 2 indicates that cantilever torsion will vanish when the negative of the measured slope $dZ/dX$ equals $\mu$, independent of the contact force. In the friction loop data of Figure 12a we see that this condition is approximately reached at the location designated by the vertical line. This remained true throughout the range of contact forces in which no wear resulted, consistent with the predictions of our model. At the above location, $dZ/dX \approx -0.1$, giving $\mu \approx 0.1$ for the transformed layer; this in turn provides a calibration of frictional force (Figure 7) via the loading force.