Scanning force microscopy of gelatin films in the dry, swollen and redried states

Greg Haugstad a, Wayne L. Gladfelter b, Elizabeth B. Weberg c, Rolf T. Weberg c, Timothy D. Weatherill c, Richard R. Jones c

a Center for Interfacial Engineering, University of Minnesota, 187 Shepherd Labs, Minneapolis, MN 55455, USA
b Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA
c Medical Products Division, E.I. du Pont de Nemours and Company, Inc., Brevard, NC 28712, USA

Abstract

We employ scanning force microscopy (SFM) to investigate films of the complex biopolymer gelatin. We distinguish different phases of gelatin via frictional force signatures. A low-friction minority phase is substantially present in thin dry films prepared from low-concentration aqueous solutions. Frictional force is measured as a function of tip speed comparatively on the different phases and related to the degree of "crystallinity" (triple-helical content) in the different film regions. The mechanical properties and surface forces are characterized in force vs. distance measurements on thick, water-swollen gelatin films prepared with and without covalent crosslinking agents. SFM imaging of redried thick films reveal "craters", which were absent in the original film. These craters are increasingly prevalent as a function of increased drying rate; an anisotropic distribution results from extremely fast directional drying. In some cases craters are observed only along boundaries between dissimilar friction domains, suggesting that interfacial stresses between domains may yield the formation of crater defects. Topographic SFM images display substantial reticulation in redried films containing a peptide coupler crosslinking agent.

Keywords: Drying; Gelatin; Scanning force microscopy

1. Introduction

Following its invention [1], scanning force microscopy (SFM) quickly established itself as a premier tool for imaging many classes of material structures down to the nanometre scale [2]. More recently, the ability to probe material properties relevant to practical applications, e.g. adhesion and tribology, has been demonstrated on model systems [3-5]. It is our observation, however, that SFM investigations of complex materials in general, and biomaterials in particular, have largely focused on topographic imaging [6,7]; the broader capabilities of SFM have yet to be exploited fully. In the present study we employ SFM measurements of topography, frictional force, and long- and short-range surface forces to investigate the structure and properties of complex biopolymer films of great importance to the photographic industry: gelatin films. Gelatin is derived from collagen and comprises the binding matrix in which light-sensitive silver halide crystals are suspended in photographic media [8]. Our intention here is not to present a comprehensive study of gelatin films, but rather to demonstrate SFM methods which can yield practical information on such complex systems.

We present results for dry gelatin films ranging from ~1 nm to microns in thickness, prepared on mica and polyester substrates. In the thinnest films on mica we image in air two distinct phases of gelatin distinguished by their frictional signature and morphology. We further differentiate the phases via the rate-dependence of sliding friction and the effects of frictional heating. In water we perform force vs. distance measurements to (1) compare the mechanical stiffness of swollen gelatin films prepared with and without a covalent crosslinking agent, and (2) investigate long-range, electrostatically derived forces during approach and complicated "stick-slip" behaviour during withdrawal. After redrying, imaged gelatin films contain crater-shaped defects or reticulation, depending on the rate of drying and the presence of intrinsic crosslinking agents. Frictional imaging elucidates the origin of the crater defects.

2. Experimental details

The Nanoscope III SFM (Digital Instruments, Santa Barbara, CA) was used, employing the 12311 scanner with lateral/vertical scanning ranges of 150/4.7 μm. Triangular
microfabricated commercial cantilevers 100 and 200 μm in length were used (quoted spring constants of 0.58 and 0.06 N m⁻¹) with pyramidal Si₃N₄ tips. Topographic and frictional force images were simultaneously collected at constant vertical cantilever deflection; the total loading force was controllably in the ~1-100 nN range, as characterized in measurements of force vs. sample displacement towards and away from the SFM tip [3]. Images were collected with the tip scanning left to right in the sample’s inertial frame, i.e. by scanning the sample right to left relative to the fixed tip in the laboratory frame. Friction-actuated cantilever torsion was enabled by choosing a fast scan direction perpendicular to the primary cantilever axis. 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. Frictional force, being non-conservative, was quantified as the line integral over a closed left to right, right to left loop.

Thick (micron scale) gelatin films were prepared from an 8 wt.% aqueous solution of gelatin (Kind and Knox photographic grade, type 2688) on polyester substrates (E.I. du Pont de Nemours, Inc., Medical Products Division, Brevard, NC) using a hand bar coater. Formaldehyde was added in some cases (as noted in the text) just prior to coating to provide amine-to-amine coupling; a proprietary, patented DuPont peptide coupler was added in other cases. Gelatin films of thickness of the order of nanometres were adsorbed over 3 h onto freshly cleaved muscovite mica (Union Mica Corp.) from aqueous solutions of 10⁻³ to 10⁻² wt.% Details of gelatin solution preparation and sample extraction/drying for these thin films are described elsewhere [9].

3. Results and discussion

In Fig. 1 we present 35 000 × 35 000 nm images of surface topography (left) and tip-sample frictional force (right) collected in air simultaneously on a thin gelatin film prepared on mica from 10⁻³ wt.% aqueous gelatin solution [9]. Brighter contrast corresponds to higher elevation or frictional force. The images reveal one large and several smaller elevated surface regions, roughly circular in shape, and a lower surrounding surface which exerts a higher frictional force on the SFM tip. We have analysed extensively such results and reported our findings in another article [9]: the elevated regions are 1.5 nm thick islands which rest on top of a 1-4 nm thick gelatin layer, the latter completely covering (wetting) the mica substrate. We have attributed the low-friction phase to moieties of triple-helical (diameter = 1.5 nm), intramolecularly folded gelatin, and the high-friction layer to the gelatin network containing both triple-helical physical crosslinks ("crystalline" gelatin) and looser amorphous regions of polypeptide strands [10,11].

More recently we have investigated the velocity dependence of frictional force comparatively on the two phases [10]. Representative results are shown in Fig. 2. The velocity v was varied via the scan length X at scan frequencies f of 55 Hz (open circles) and 5 Hz (open triangles) on the high-friction layer and at v = 55 Hz on a large, low-friction island (closed circles); note v = 2Xf. A frictional increase at smaller scan lengths/velocities, reflecting the onset of rubbery behaviour induced in part by local frictional heating [12], is observed on the high-friction network but not on the low-friction islands. After "scan heating" a small network region, larger images taken at non-perturbative scanning conditions revealed elevated friction in the affected region; however, no corresponding topographic changes were observed. We assign the greater frictional dissipation of energy to heightened molecular relaxation in the rubbery regime. Raising the loading force above some critical value on the network film indeed results in the extensive rearrangement and flow of gelatin [9], presumably due to scan-induced "melting". Scanning above a critical load on the islands also results in melting, but without first passing through a regime of rubbery behaviour. This is consistent with our identification of the islands as moieties of triple-helical gelatin, lacking the loose amorphous domains in which net stretching can occur. This example suggests that important phase behaviour in a com-

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Fig. 1. SFM images (35 000 × 35 000 nm) of surface topography (left) and tip-sample frictional force (right) collected in air simultaneously on a thin gelatin film, focusing on large and small islands. Brighter contrast corresponds to higher elevation or frictional force.

Fig. 2. Velocity dependence of frictional force on the low- and high-friction phases of gelatin. The velocity was varied via the scan length at scan frequencies of 55 Hz (open circles) and 5 Hz (open triangles) on the high-friction layer and at 55 Hz on a large, low-friction island (closed circles).
Further, the extent of penetration is substantially less on a film that was covalently crosslinked with formaldehyde (75 mM per 200 g gelatin), compared with one with solely physical (intrinsic) crosslinks [10]. These observations presumably reflect differences in elastic modulus. Characterization uniquely to SFM would be to map out possible lateral variations in modulus across these films.

Fig. 3(b) focuses on weak forces sensed in water while approaching a relatively thin gelatin film (~10 nm when dry). The data are representative qualitatively of results obtained on thicker films as well. We generally find exponentially repulsive forces measurable up to distances of order 100 nm (note logarithmic force scale); the distance scale was zeroed at the point of departure from this exponential trend. The repulsive long-range forces presumably reflect a Debye screening profile set up by ionized species in response to the charged gelatin surface [13] (measurements on clean mica surfaces in water displayed weaker forces of much shorter range). Thus employing simple SFM measurements the ionicity of a water-swollen polymeric film, with or without ionizing additives, can be assessed on the nanometre scale.

Fig. 3(c) reveals attractive forces sensed in water up to micron-scale distances during withdrawal from a formaldehyde-crosslinked film; here the broad distance scale was zeroed at the location of zero force. We observe the gradual build-up and random, partial release of attractive forces with increasing distance. The sudden vertical jumps in force ~0.05–0.30 nN in magnitude are similar to those observed by others investigating biological interactions in simpler systems [14]. We assign the vertical jumps to slippage of gelatin strands past one another, i.e. the failure of attractive interactions of strength on the order of several hydrogen bonds. We have not yet attempted to compare in detail this behavior in films with different molecular coupling (e.g. extrinsic vs. intrinsic crosslinking); statistical analysis will probably be required [14]. These preliminary results suggest however that interactions on the scale of single biological adhesive forces can be sampled easily with SFM on complex systems.

In Fig. 4(a) we present a 9000×9000 nm topographic image of a thick gelatin film after exposure to a drop of water and fast drying. The drying was performed by touching a paper towel to the drop, being careful not to touch the film itself; this immediately removed all visible water from the gelatin surface at the location subsequently imaged. The film images contain an anisotropic distribution of craters on the order of 10 nm deep and ~100–1000 nm in diameter, as well as taller particulates of material, presumably gelatin. No such features were present on the film prior to water exposure. Fig. 4(b) contains 15 000×15 000 nm topography/frictional force (left/right) images of a film dried much more slowly, allowing the water drop to evaporate in ambient conditions. Here a smaller number of crater defects and particulates are observed, but of similar size to those in Fig. 4(a). Relatively large frictional forces are sensed in these craters, suggesting some modified chemical character [4]. Particularly remarkable is the presence of two distinct levels of friction on the
surface away from the craters: close inspection reveals that the craters form along the boundaries between the two dissimilar regions. We tentatively attribute crater formation to interfacial stresses exerted between these regions.

Fig. 5 contains preliminary 50,000 × 50,000 nm topographic images of thick gelatin films prepared with the peptide coupling crosslinking agent at concentrations of (a) 23 mM and (b) 60 mM per 200 g of gelatin, following exposure to a water drop and ambient drying. We find no evidence of crater formation but observe instead a number of dome-shaped protrusions absent in the original film, with size and number density differing for different crosslinker concentrations. In Figs. 5(a) and 5(b) typical protrusions are respectively ~3000 nm and ~500 nm in diameter, ~25 and ~10 nm in height, and of number density ~2 × 10⁶ cm⁻² and ~1 × 10⁷ cm⁻². This morphology is apparently due to reticulation, which results from variations in local hardening in the gelatin films [8]. At higher concentration a larger number of smaller, more closely spaced protrusions are observed.

In sum, the images in Figs. 3 and 4 show how complex polymeric systems with broad molecular weight distributions and added crosslinking agents can exhibit singular, characteristic behaviour under particular conditions, and how routine SFM analysis can provide insight into defects formed during processing.

### 4. Summary

Thin gelatin films prepared from a low-concentration aqueous solution contained two distinct phases distinguished by frictional forces. The low-friction moieties were thought to contain only triple-helical (collagen fold) gelatin which exhibits reduced molecular relaxation, and hence frictional dissipation, relative to the dominant network film. The rate dependence of frictional force unveiled different phase behaviour and suggested that the presence or lack of rubbery characteristics can be assessed with SFM, and in turn related to the presence or lack of loose amorphous regions in the polymer film. In water, the mechanical stiffness, a function of intermolecular coupling, was gauged qualitatively in force vs. distance measurements. In addition the ionicity of the film was probed locally by profiling exponentially repulsive forces in the non-contact regime. In the contact regime weak intermolecular interactions were manifest in small (<1 nN), sudden releases of attractive force during withdrawal. Redried gelatin films displayed crater defects whose number increased at more rapid drying conditions. Directional drying yielded an anisotropic distribution of craters mirroring this direction. At more moderate drying conditions the resulting craters apparently formed along boundaries between characteristically different gelatin film domains, imaged via frictional forces. The use of a peptide coupler crosslinking agent resulted in extensive reticulation with geometric parameters reflecting the concentration of the coupler.
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References