Submicroscopic Morphological Changes of Laser Induced Surface Structure of Collagen, Tendon, and Gelatin Films

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The formation of nap-type or wall-type structures on laser irradiated collagen related biopolymers films is reported. An atomic force microscope (AFM) allows a highly sensitive surface characterization of the same area of the irradiated sample between subsequent laser pulses. A change in surface topography was found to be dependent on the fluence and number of applied pulses. A successive measurement technique of AFM gave significant information on the mechanism of surface structure formation.

Excimer laser ablation has become a proven and valuable technique for material treatments like cutting and surface modification.[1–3] Some researchers reported various types of surface structures produced by laser irradiation on polymer films.[4–6] Several authors have described a structuring effect on polymers which were related to the cooperative material transport driven by tension fields and laser induced temperature fields.[6,7] This mechanism was ascribed to thermal denaturation which developed within a thin molten surface layer and the subsequent release of frozen-in tension fields. However, the initial stage of the evolution of the surface structure and material change still remains uncertain.

We have so far investigated the formation mechanism of surface structures for collagen films by using atomic force microscope (AFM).[8,9] The AFM has a superior spatial resolution compared to a scanning electron microscope (SEM) and is a powerful technique for studying the surface morphology of various polymers after laser irradiation. With regard to the surface structures, the AFM investigation of the correlation between laser pulses in the same area and the data related to surface roughness indicates a complicated change in the surface. The successive measurement technique is useful for revealing the mechanism of microstructure formation. In this study, we demonstrated that molten surface layers and thermal denaturation were necessary for structure formation using collagen films and also demonstrated imaging of the characteristic changes within the same surface area from pulse to pulse.

Collagen films were prepared by the cast method using type I atelocollagen (KOKEN) extracted from a calf skin and the film thickness was about 70 μm. Tendon films were prepared by slicing the calf tendon. Gelatin was prepared by denaturing the collagen. Gelatin films were prepared by casting 2% aqueous gelatin at 60 °C, followed by the evaporation of water. The laser used was an ArF (193 nm) excimer laser (LEXTRA50, Lambda Physik) whose pulse duration in FWHM was 17 ns. The AFM used was a Nanoscope III (Digital Instruments) and operated in the contact mode under atmospheric conditions for films observation. Successive AFM measurements were carried out by adjusting film placement at the same position in the scanned area with a specifically marked corner.

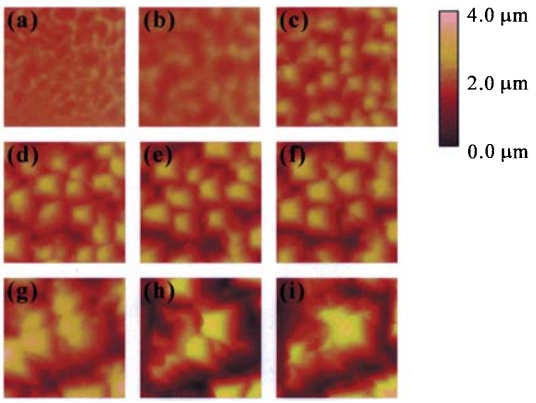

Figure 1. AFM images of collagen films irradiated with 193 nm at a fluence of 100 mJ/cm^2. (a) 5 pulses, (b) 10 pulses, (c) 20 pulses, (d) 30 pulses, (e) 40 pulses, (f) 50 pulses, (g) 100 pulses, (h) 150 pulses, (i) 200 pulses. The scan size is 5 × 5 μm.

AFM images of the surface of collagen film irradiated with various pulse shots are shown in Figure 1. The scanned area of the sample in Figure 1 is 5 × 5 μm. The results not only demonstrate the structure formation as a function of the number of applied pulses, but the successive AFM measurements also allow one to investigate changes within the same area. The untreated sample has no irregularities and no decisive damage such as scratches. The surface is very smooth and a similar topography is measurable on a number of different collagen samples. After the first laser pulse at 100 mJ/cm^2, the surface

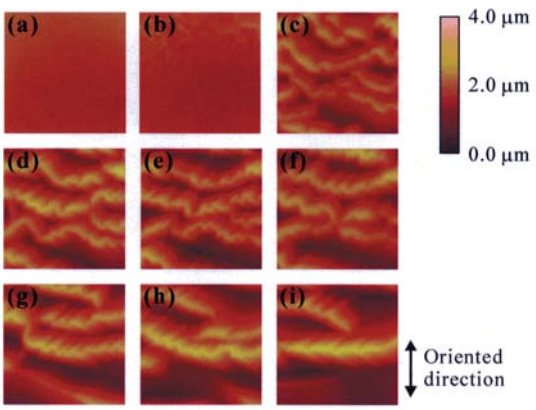

Figure 2. AFM images of tendon films irradiated with 193 nm at a fluence of 100 mJ/cm^2. (a) 5 pulses, (b) 10 pulses, (c) 20 pulses, (d) 30 pulses, (e) 40 pulses, (f) 50 pulses, (g) 100 pulses, (h) 150 pulses, (i) 200 pulses. The scan size is 5 × 5 μm.

topography had slightly changed. The topographical change observed on the irradiated surface of the films was caused by the photothermal process of laser ablation and thermal denaturation of the collagen molecules. Collagen molecules were denatured in the form of gelatin by heat, and gelatin chains were immediately shrunk, therefore, a topographical change was observed on the irradiated surface. This conversion of collagen to gelatin was confirmed by IR spectra. These detailed results will be reported somewhere. The surface structural changes were clearly discriminated after the fifth laser pulse. The treated surface is characterized by "summit" and "valley". Additional laser pulses enhanced the summit and valley structure but a transition into another topography has not been observed. The evolution of the structure was governed by its features after a few pulses. The number of the summits was decreased by increasing the number of pulses. Part of the remaining summits corresponded to the section where the summits were concentrated. Based on these results, it is suggested that the first structure was generated by thermal denaturation of the collagen, and then the increase in the size of the structure would be subjected to the dense of the summits. This suggestion is supported by the results of the tendon films described below. The top of summits would be hardened by shrinkage of gelatin chains, and therefore, the etching rate of the summit was smaller than the valley. Repeat of etching and shrinkage would induce the aggregation of the summits. The several summits, then, were transformed into a new single summit.

Similar studies were performed for the tendon films. Results of AFM imaging for the tendon film irradiated at a fluence of 100 mJ/cm^2 are shown in Figure 2. The treated surface of the tendon films, composed of collagen molecules oriented along one axis, was characterized by wall type structure, whereas for the collagen films composed of random oriented collagen molecules, summit and valley structures were observed on the irradiated collagen films. The evolution of the structure for the tendon film was similar to that observed in the collagen film. The number of walls decreased with increasing the number of pulses. Figure 3 shows the cross-sectional profiles of the wall type structures at a fluence of 100 mJ/cm^2. The height of walls and the peak-to-peak distance increased with laser pulse. This result supports the hypothesis for the structure growing process. The increase in the height would mean that the etching rate of the wall was smaller than that of the valley. The walls and the valleys were etched with laser irradiation. The increment of the etching depth at 30 pulses (for Figure 3(d)) from that at 20 pulses (for Figure 3(c)) was about 1 μm, and the height of the walls for Figure 3(d) was higher than that for Figure 3(c). As a result, a difference in the etching rate between the walls and valleys and/or the shrinkage of gelatin chains would increase the height of the walls. The increase in the peak-to-peak distance would mean that the number of walls decreased and several walls apparently converged after multi irradiations.

In order to confirm that the thermal denaturation of collagen molecule would be necessary for the formation of the structure, similar experiments were performed for gelatin films. Figure 4 shows that a topographical change was not observed on the irradiated surfaces of the gelatin films. The results in Figure 4 reveal that the thermal denaturation is responsible for the formation of the structure.

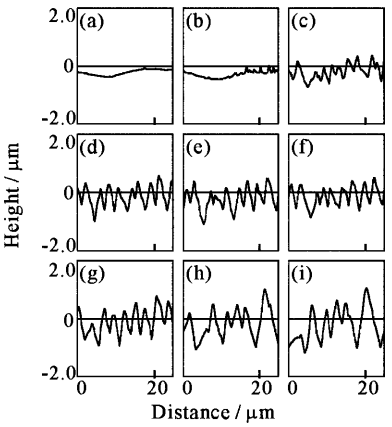

Figure 3. Cross-sectional profiles of tendon films irradiated with 193 nm at a fluence of 100 mJ/cm^2, where the distance is enlarged 5 times than that in Figure 2. (a) 5 pulses, (b) 10 pulses, (c) 20 pulses, (d) 30 pulses, (e) 40 pulses, (f) 50 pulses, (g) 100 pulses, (h) 150 pulses, (i) 200 pulses. The cross sections were measured in the oriented direction in Figure 2.

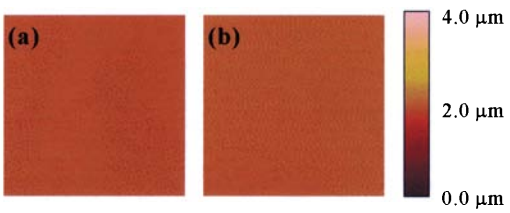

Figure 4. AFM images of gelatin films irradiated with 193 nm at a fluence of 100 mJ/cm^2. (a) 10 pulses, (b) 200 pulses. The scan size is 5 × 5 μm.

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