

Imaging of the Lectin-Labeled Cell Surface of Human Lymphocytes by the Use of Atomic Force Microscope

Motoharu SAKAUE¹⁾ and Kazuyuki TANIGUCHI^{2)*}

¹⁾Department of Veterinary Anatomy, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657 and ²⁾Department of Veterinary Anatomy, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

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ABSTRACT. The atomic force microscope (AFM) is a new useful tool to examine the surface structure of specimens with a higher resolution than the conventional scanning electron microscope. In the present study, we used the AFM to observe the surface of paraformaldehyde-fixed human lymphocytes processed for histochemistry using a biotinylated lectin, wheat germ agglutinin, followed by colloidal gold and silver-enhancement method. Before the treatment, no particles were attached to the cell surface. After treatment, many particles about 100 to 150 nm in diameter were visualized on it. Since we could observe the same cells on the slide glass before and after treatment, the AFM has the advantage to enable us the repeated imaging of samples treated with various kinds of histochemistries.

KEY WORDS: atomic force microscope, human lymphocyte, lectin-histochemistry.

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The atomic force microscope (AFM) [1], one of the scanning probe microscopes [2], is a useful tool to examine the surface structure of various substances including chemical materials [3] and biological specimens [4–6, 8–13]. In general, the AFM has two advantages as follows. First, it can get images from samples in any conditions [1], even in water [4, 5, 9, 11, 12]. Second, it can examine samples theoretically with a higher resolution than the conventional scanning electron microscope (SEM) [7, 13]. These advantages force the investigators to apply the AFM to the field of cell biology, because the AFM is expected to give the images of structures such as cell surface receptors, channels and carbohydrate chains [7]. In this context, it is necessary to examine the cell surface before and after treatment with various histochemical methods to compare the influence of treatments to cells. Such repeated examination is almost impossible by the use of the conventional SEM, because it needs various pretreatments to observe specimens in vacuum. On the other hand, the AFM can get images from almost intact samples in any conditions as described above, although there has been no report on the condition of the same cell before and after treatment with a certain histochemical method. In the present study, therefore, we examined the cell surface of human lymphocytes by the use of AFM before and after treatment of lymphocytes with histochemistry using a biotinylated lectin followed by colloidal gold and silver-enhancement method to detect the positive reaction of the treatment on the cell surface.

Lymphocytes were isolated from heparine-anti-coagulated human venous blood according to the Ficoll-paque plus (Amersham Pharmacia Biotech, Uppsala, Sweden) density gradient methods. They were suspended in 0.1 M phosphate-buffered saline (PBS, pH 7.6) and dropped on a

silan-coated slide glasses to make smear samples. The smear samples were fixed by acetone followed by 0.1 M PBS containing 4% paraformaldehyde for 2 min at room temperature, and washed in distilled water three times. The samples were then dehydrated through acetone and air-dried at room temperature. The surface of lymphocytes was scanned in air with a NanoScope™ IIIa AFM (Digital Instruments, Santa Barbara, CA, U.S.A.) by contact mode at a scan rate of about 2 Hz and with V-shaped Si₃N₄ cantilevers (Digital Instruments) at spring constant of about 0.06 or 0.12 N/m. It took about 2 min to obtain one image because of 256 scan lines per an image. After imaging, the smear samples were immersed in 1 % bovine serum albumin for 30 min at 32°C to block non-specific reactions, incubated with biotinylated wheat germ agglutinin (WGA, 1:1,000, Vector Laboratories, Burlingame, CA, U.S.A.) for 2 days at 4°C and treated with avidinylated colloidal gold particles (25 nm in diameter, 1:500, E-Y Laboratories, San Mateo, CA, U.S.A.) for 40 min at 32°C. Thereafter, the silver enhancement method was applied to the samples for 10 min at 4°C according to manufacturer's instruction (British Biocell International, Cardiff, UK). Then, the slides were dehydrated through acetone, air-dried, and re-observed with the AFM in the same way described above. In the control staining, the samples were incubated with PBS instead of WGA, and reacted with colloidal gold followed by the silver enhancement method. No positive signal was observed in the control staining.

Lymphocytes observed with the AFM before the treatment with lectin-histochemistry were round or elliptical in form and 8 to 10 µm in diameter (Fig. 1A). The cell surface of lymphocytes was almost smooth and no particles were attached to the cell surface. After treatment with lectin-histochemistry followed by silver-enhancement method, the positive signals were detected with the AFM as a lot of particles on the cell surface of the same lymphocytes previ-

* CORRESPONDENCE TO: Dr. TANIGUCHI, K., Dept. of Veterinary Anatomy, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan.

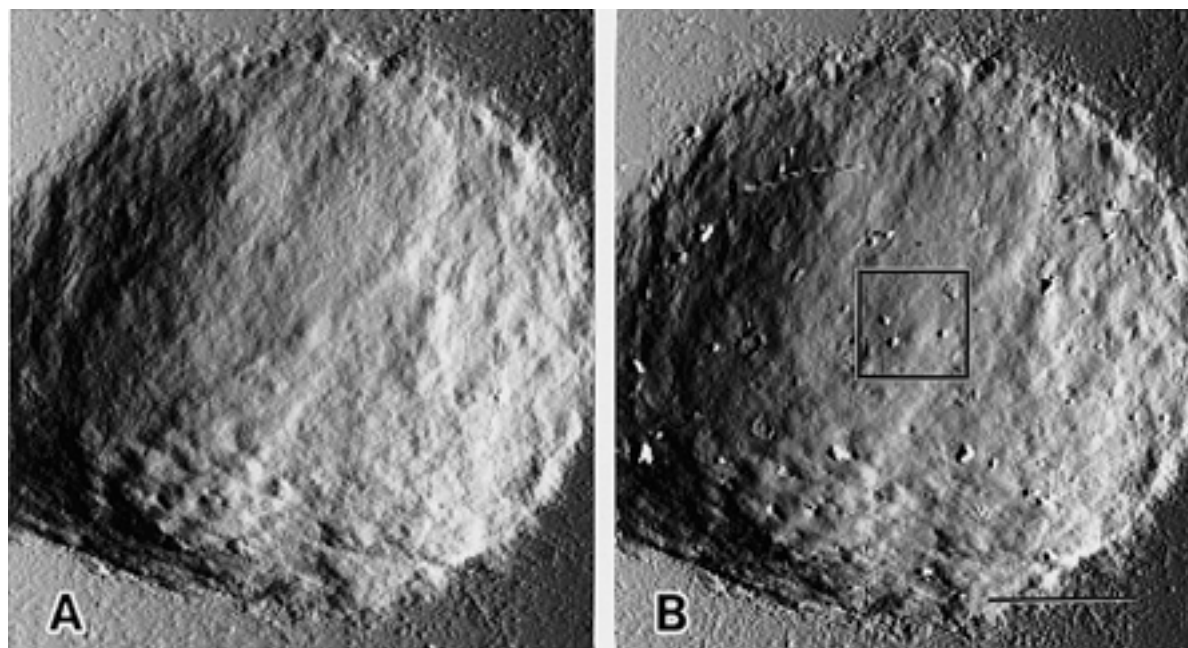


Fig. 1. AFM images of a human lymphocyte before (A) and after (B) the histochemistry. By comparison of A and B, the positive reaction on the cell was more clearly identified as dot-like structures. The open square on B indicates the scan area displayed in Fig. 2. Bar = 2 μm .

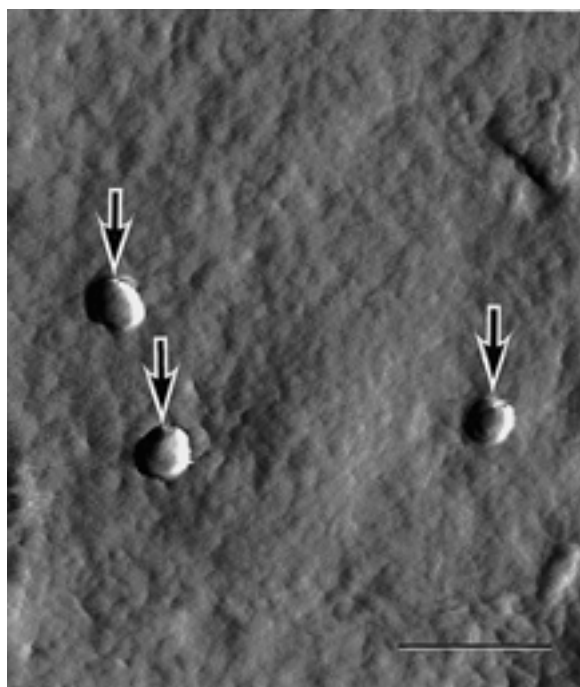


Fig. 2. The AFM image at high magnification from the open square in Fig. 1B. Positive signals (arrows) were clearly visualized on the cell surface. Bar = 0.3 μm .

ously observed without lectin-histochemical treatment (Fig. 1B). At the higher magnification, the diameter of particles was calculated as about 100 to 150 nm (Fig. 2). Particles about 300 nm in diameter were occasionally encountered. They seemed to be the aggregation of several gold particles. The three-dimensional image (Fig. 3) reconstructed by calculation from data on Fig. 2 made positive signal, gold-colloids, clearer and gave fine figure of the cell surface. Additionally, the height of those signals was 50 to 60 nm calculated from Fig. 3.

The present study proved that the AFM is quite useful to detect cell surface signals undetectable by light microscopy. The AFM gave us the detailed three-dimensional data about cell surface signals with only one time of imaging at higher magnification than the scanning electron microscopy (Figs. 2 and 3) and probably enable to measure the length or height of signals on the cell surface. By light microscopy, the vertical resolution of samples is theoretically difficult. By conventional scanning electron microscopy, it is almost impossible to obtain data on the cell surface before and after histochemical treatment successively, because the first treatment to examine specimens with the SEM in vacuum eliminates the histochemical reactivity on the cell surface. The SEM generally requires some metal-coatings on biological samples, and cannot observe the real structure of the surface. On the other hand, the treatment of samples for the observation with the AFM gives less damage to samples to preserve the histochemical reactivity after the first observation with the AFM. The particles shown in Fig. 1B are proved to be the positive reaction product of lectin-his-

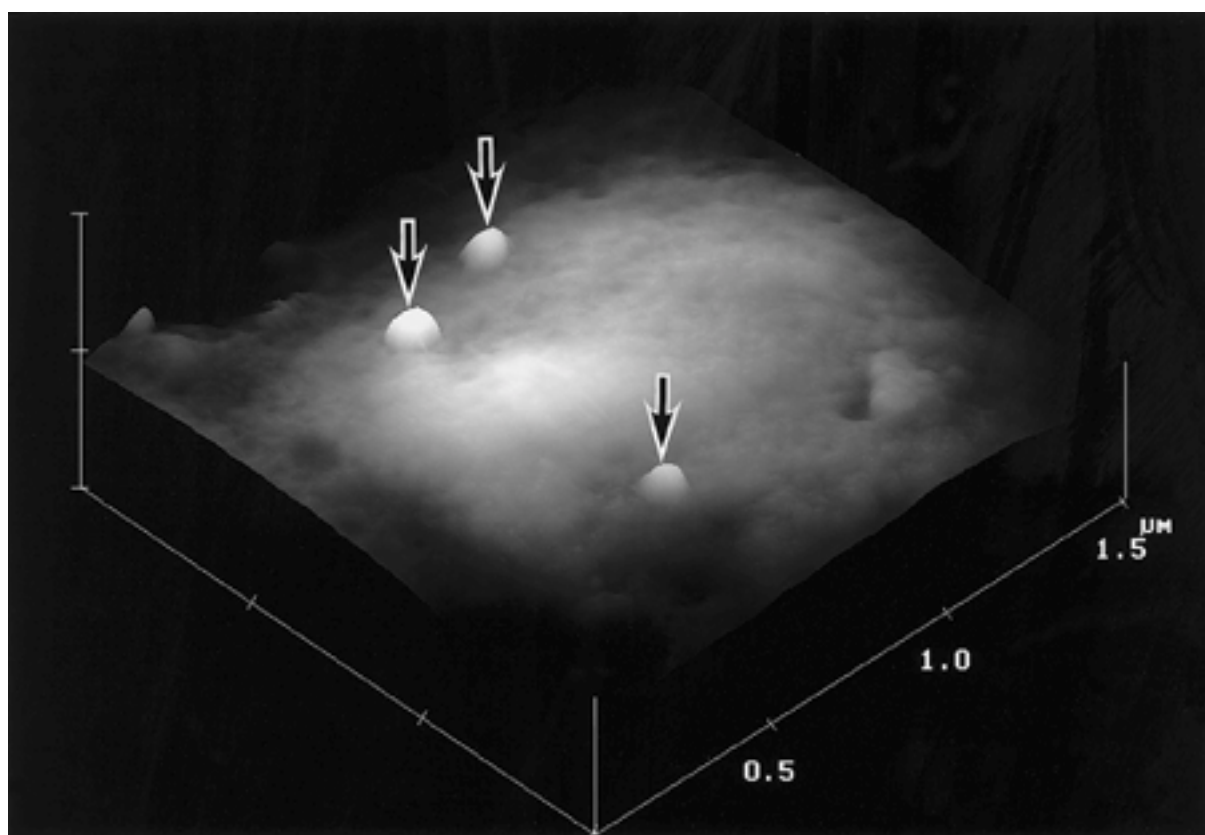


Fig. 3. A three-dimensional AFM topographic image reconstructed by calculation from data on Fig. 2. Three positive signals can be more clearly observed on the cell surface of the lymphocyte (arrow). The scale of horizontal sides, $1.5 \mu\text{m}$; that of vertical side, $0.5 \mu\text{m}$.

tochemistry by the comparison of the same cell before and after lectin-histochemical treatment. In addition, the real surface of the cell is imaged by AFM because of no need of any metal coatings.

In short, we tried to apply the AFM to the analysis of cell surface before and after lectin-histochemical treatment successively, and revealed for the first time the image of the same cell surface before and after treatment with a higher resolution than the conventional SEM. The present results proved that the AFM is a reliable tool to detect specific materials on the cell surface precisely. In addition, the present method of lectin-histochemistry followed by colloidal gold and silver-enhancement method may be quite useful for the analysis of distribution of molecules even on the living cell surface.

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