SCANNING FORCE MICROSCOPY INVESTIGATIONS OF HUMAN PLATELETS

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In the research surface morphology of resting platelets and platelets after exposure to ADP was investigated by SFM. Here we demonstrate that SFM offers a unique opportunity to visualize with high spatial resolution on a submicron scale a drastic surface morphology changes of the platelets after exposure to ADP.

1. Introduction

The scanning force microscope (SFM) is becoming a powerful instrument that can image the surface morphology of biological samples in various (air, liquid, vacuum) environments with high spatial resolution on a nanometer scale [1]. Examples of SFM applications to image living cells under physiological conditions in a nondestructive manner are a human platelets during their activation [2], the enzymatic activity detection [3], the observation of exocytotic events in living cells [4]. One of the exclusive features of the SFM is the ability to image not only topography but also to obtain viscoelactic information of biological samples. Examples are measuring the elastic properties of platelets [5, 6], embryonic carcinoma cells [7], glial cells [8].

In our research the platelet surface morphology changes were investigated by SFM after addition of 10^{-5} M ADP to cause platelet aggregation. Platelets are the smallest cellular elements of blood in 2.5 μm in average normal diameter. They play a key role in haemostasis and normally circulate in a resting state. They aggregate upon activation, forming a thrombus which serves to plug the loss of blood from damaged blood vessel. General functional properties of blood platelets include adhesion, aggregation, release, clot formation. These functional expressions usually are associated with a drastic surface morphology changes of platelets. A number of approaches have been used to evaluate platelet adhesion, aggregation, and secretion, and within the last 10 years much interest has been directed to the biochemical mechanisms and signal transduction events occurring during these various phases of function [9]. The scanning force microscope holds great promise as an instrument to visualize surface morphology changes occurring immediately after platelet activation [2, 5].

2. Experimental

Platelet rich plasma was prepared by centrifugation of the blood at 200g for 10 min with an OPN-03 centrifuge (Laboratory Equipment and Devices, Russia). Resting and activated platelets were prepared by fixing platelet suspensious in 1,5% glytaral-

dehyde for 30 min. Then they were attached to mica by centrifugation at 250g for 30 seconds. Platelets were washed five times in 0,2 M Na_2HPO_4/NaH_2PO_4 saline buffer. The fixed platelets were then dehydrated by 5 min incubations in 30%, 50%, 70% and 96% ethanol and were critical point dried. To cause platelets activation 10^{-5} M ADP was used.

Surface morphology of resting platelets and platelets after exposure to ADP was investigated by a commercial SFM (Digital Instruments, Nanoscope (R) IIIa, Dimension 3000; NT-MDT, Solver-P47). Images were captured in air using tapping-mode SFM. Commercially available, 123–μm-long silicon cantilevers with spring constants of 29- 57 N/m were used for most experiments (Nanosensors GmbH).

3. Results and Discussion

Fig.1. shows the resting platelets. The resting cells are relatively flat and exhibit discoid shape. The width of the resting platelet is about 2.5 μm and height is 660 nm.

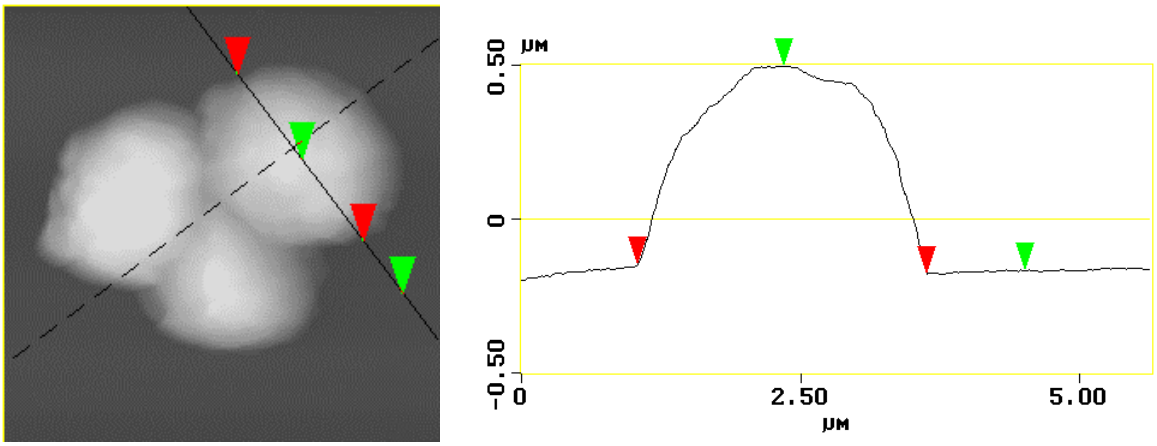

Fig. 1. SFM image of fixed with glutaraldehyde human resting platelets on mica (a); – profile along a line cutting through cell (b)

When platelets are activated in suspension with 10^{-5} M ADP they exhibit a dramatic cell shape changes. The SFM image of the activated platelets is given in Fig. 2. The width of the activated platelet is about 2.4 μm and height is 1.1 μm.

One of the most striking morphological changes that occur in the platelets after exposure to ADP is rapid appearance of multiple surface projections termed pseudopods. Long (~1-5 μm), thin with width of 130 nm and height of 35 nm, a slight tapered pseudopods extend from the discoid cell body (Fig. 2,3). Platelets pseudopods have generally been considered important in platelet aggregation. The main pseudopods function is to bind fibrin and other platelets to form a three dimensional clot. They also increase collision frequency of platelets. The topographical orientation of pseudopods is not random and represents a topography that optimizes the ability of the platelets interaction (Fig. 3).

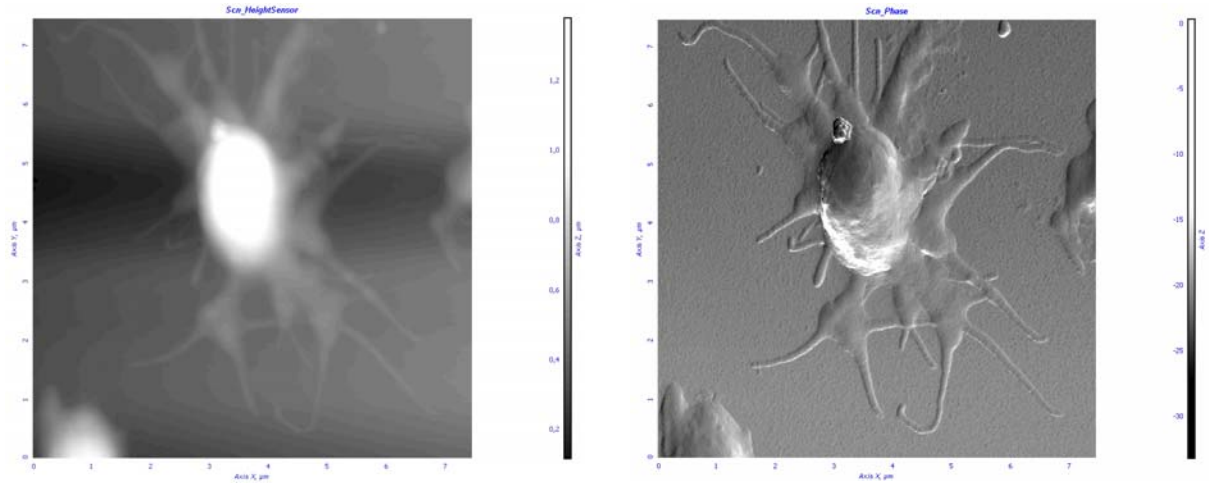

Fig. 2. SFM image (height and phase) of activated with 10^{-5} M ADP human platelet fixed with glutaraldehyde

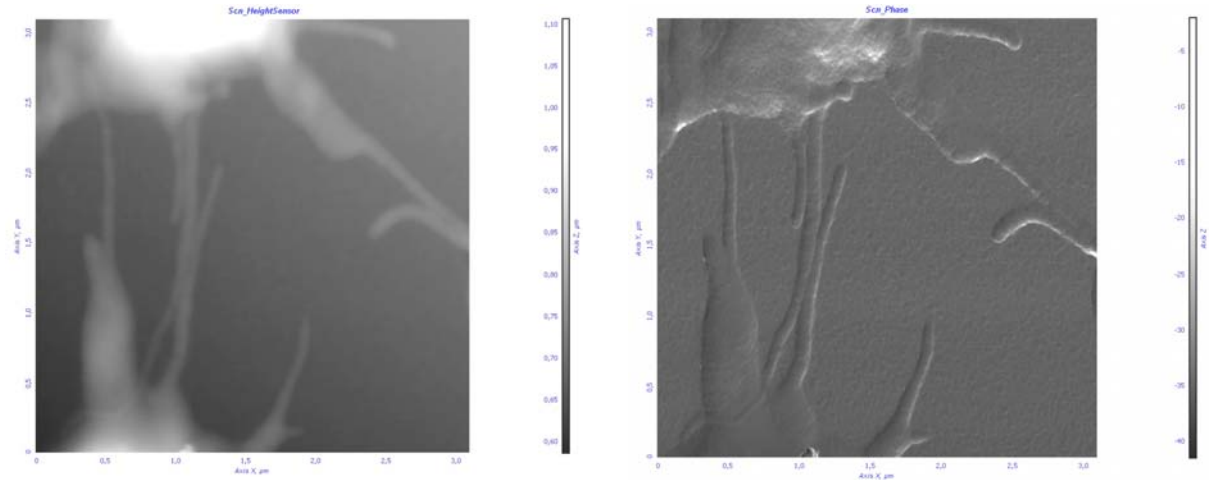

Fig. 3. SFM image (height and phase) of pseudopods of human platelets activated by 10^{-5} M ADP

Long pseudopods containing elongated bundles of actin filament are shown in Fig 3. The length of a pseudopods vary from 1 μm to 5 μm. The width of the pseudopod is about 140 nm and height is 35 nm. Zooming in on the pseudopods the periodical chain-like structure with irregular size of from 30 to 40 nm is visualized (Fig.4).

The decreasing of scan area allows to observe fiber structure with the width of ~ 3 nm on the pseudopod surface (Fig. 5).

Finally, our results demonstrate that SFM offers a unique opportunity to visualize with high spatial resolution on a submicron scale the surface morphological and structural changes, which accompany the activation process of human platelets.

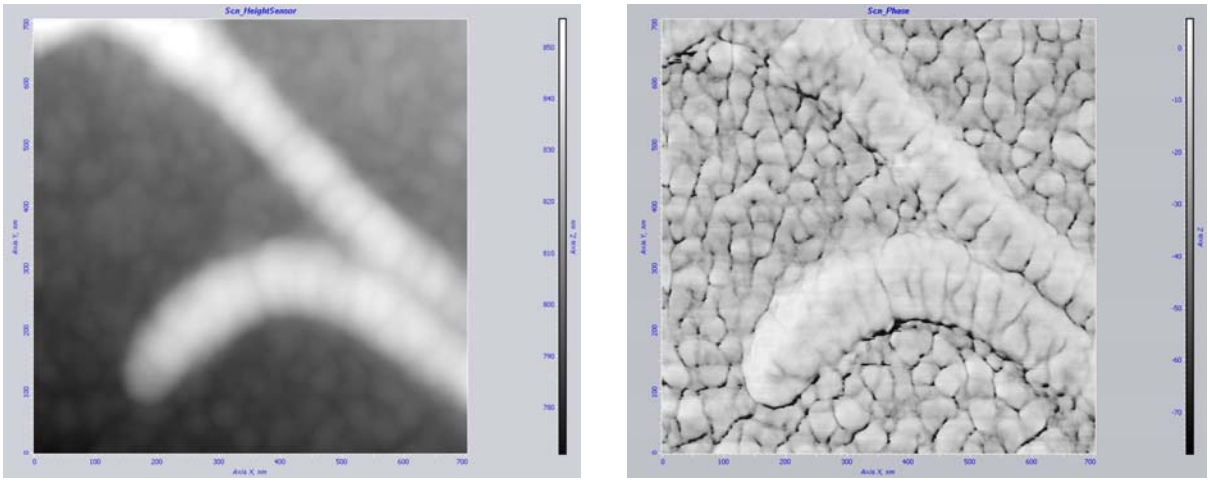

Fig. 4. SFM image (height and phase) of pseudopods of human platelets activated by 10^{-5} M ADP

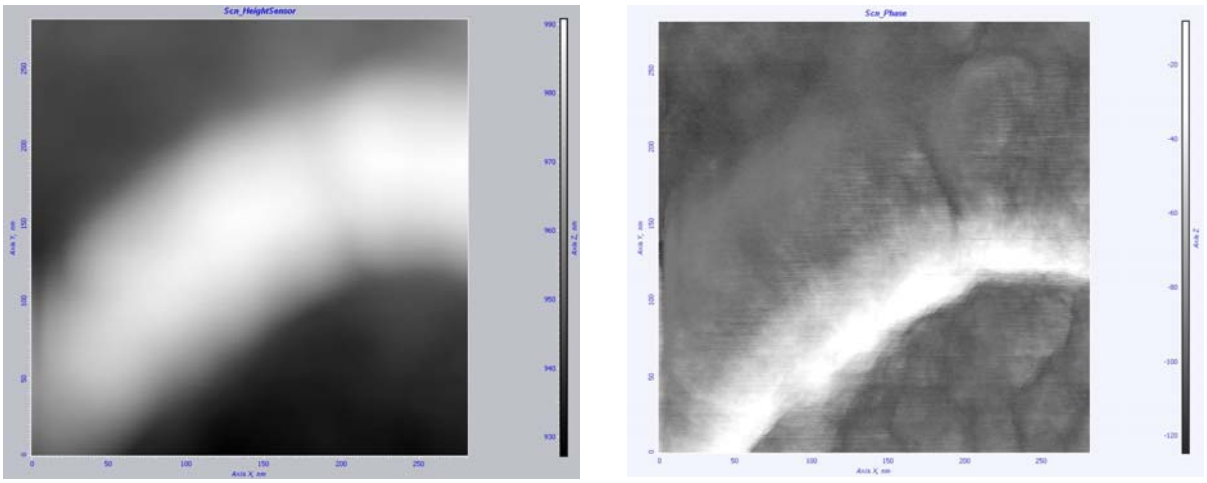

Fig. 5. SFM image (height and phase) of a pseudopod of human platelets activated by 10^{-5} M ADP

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