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SURFACE MORPHOLOGICAL CHANGE INVESTIGATION WITH SCANNING FORCE MICROSCOPE IN SURFACE ACTIVATED PLATELETS

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Reported is the surface morphology of resting and activated human platelets after adhesion events to mica by SFM. SFM investigations of the human platelet activation from the initial formation of filopodia to the fully spread form reveal details of the membrane spreading process. It was shown that platelet adhesion and spreading to mica involve the formation of two different actin-based structures, filopodia and lamellipodia, and the increase in area of platelet surface. Some common scenarios of spreading process of mica-activated platelets were recognized.

1 Introduction

A scanning force microscope (SFM) has become an extremely important instrument in cellular biology in recent times. The STM has been widely used for imaging biological cells because of the combination of high resolution (in a nanometer scale) and the ability to obtain time-dependent dynamic information about biological systems under physiological conditions. In addition to this, elastic properties of biological cells can be detected with the SFM [1–3].

Here we describe the surface morphology of resting and activated human platelets after adhesion events to mica by SFM. Platelets are the smallest cellular elements of blood in 2.5 μm in average normal diameter that normally circulate in a resting state, exhibiting a discoid shape. They play a key role in hemostasis and thrombosis. Platelet activation takes place after attachment and adhesion events or after addition of soluble agonists that trigger activation mechanisms. Upon activation platelets undergo a dramatic cell shape changes from a flat discs of 2.5 μm in diameter to the fully spread ("fried-egg") platelets of 8–10 μm in diameter. During their activation a drastic reorganization of their cytoskeleton, a secretion of intracellular granules contents, an expression of the receptors for fibrinogen and coagulation factors, an aggregation occur. The aim of the present study was to investigate the surface morphology change of platelets activated by adhesion to mica with SFM.

2 Material and method

Venous blood was collected from healthy donors. Platelet rich plasma (PRP) was obtained by centrifugation citrated blood at 110 g for 10 min at room temperature. The platelet suspension was incubated at 37 °C for 30 minutes before the mica adherence

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studies were performed to allow platelets to reach a resting condition. Mica-activated platelets were prepared by micropipetting of suspended platelets onto the mica surface and allowing them to settle for 30 seconds, 1 minute, 4 minutes, 10 minutes. A drop of 1.5% glytaraldehyde was placed on mica substrates for fixation of mica-activated platelets for 30 min. Then platelets were washed five times in HEPES saline buffer, dehydrated in a graded series of ethanol and critical point dried.

All data were obtained on a Nanoscope (R) IIIa MultiMode atomic force microscope (Digital Instruments, Santa Barbara, CA). E and J scanners were used. Images were captured in air using tapping-mode SFM with commercially available 123-µm-long silicon cantilevers (spring constants of 29–57 N/m, Nanosensors GmbH). SFM images were processed with the Nanoscope software (Digital Instruments/Veeco).

3 Results and discussion

For SFM study of resting platelets one part of the platelet suspension was incubated at 37 °C for 30 minutes before addition of glytaraldehyde fixative to allow platelets to return their discoid shape. Another one was not preincubated before glytaraldehyde fixation. The incubated resting platelets fixed and then adhered to mica were relatively flat and exhibit discoid shape with width ~2.5 µm and height ~300 nm (Fig. 1).

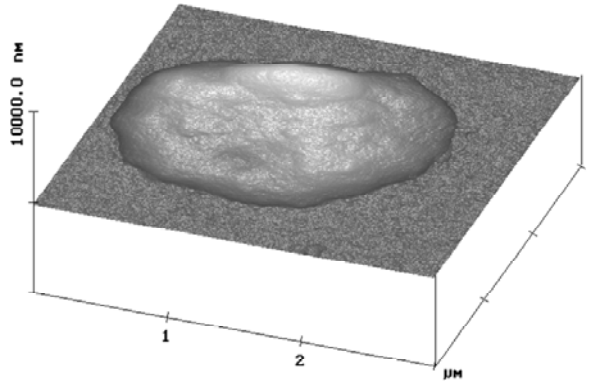

Fig. 1. SFM image of human resting platelet on mica (discoid shape)

Non-preincubated resting platelets showed a considerable range of morphologies from disks to disks with protuberances with width ~2.3 µm and height ~400 nm (Fig. 2 a), spheroid shape with long, thin filopodia (Fig. 2 b, c), spindle-shape forms with width ~2.4 and ~1.3 µm and height ~560 nm. Figure 2 b, c shows that thin with width ~180 nm and height ~60 nm, a slight tapered filopodia extend from spheroid cell body. The filopodia length varies from 1µm to 3.5µm. Most likely, this shape distribution was the result of the preparative procedures without pre-incubation of platelet suspension before glytaraldehyde fixation.

When platelets were mica-activated they exhibited a dramatic cell shape changes. The most striking features of the mica-activated platelets surface morphology were the formation of two different actin-based structures, filopodia and lamellipodia, and the increase in area of platelet surface.

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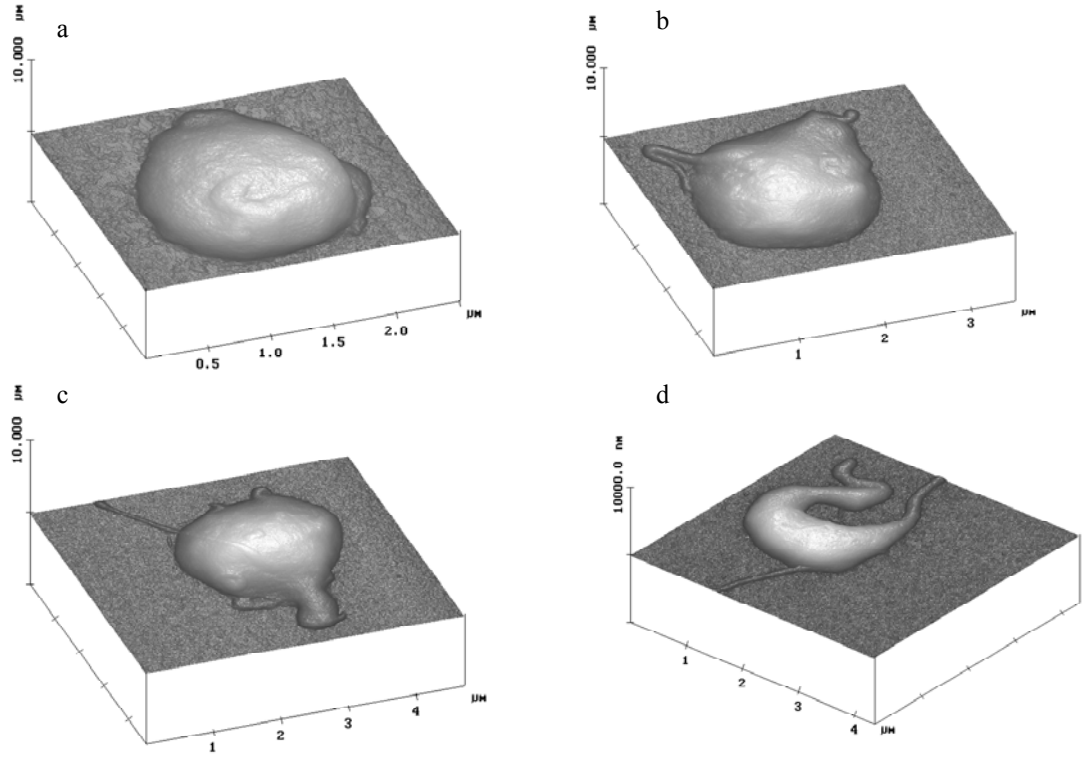

Fig. 2. SFM images of human resting platelet on mica: a – disks with protuberances; b, c – spheroid shape with short, stubby filopodia and long, thin one; d – spindle-shape

Platelets in the initial stages of adhesion to mica produced several long filopodia extending over the substrate. The irregular protrusions at platelet margins and blunt filopodia were also observed (Fig 3, a). Figure 3, a shows that the platelet has more rounded shape with width of 2.2 μm and height of 600 nm. Zooming in on the filopodia the periodical chain-like structure with irregular size of from 40 to 55 nm was visualized (Fig. 4, a). It should be noticed that extension of filopodia is powered by polymerization of the structural protein actin into filaments. Filopodia contain elongated bundles of actin filaments. The main filopodia function is to bind fibrin and other platelets to form a three dimensional clot.

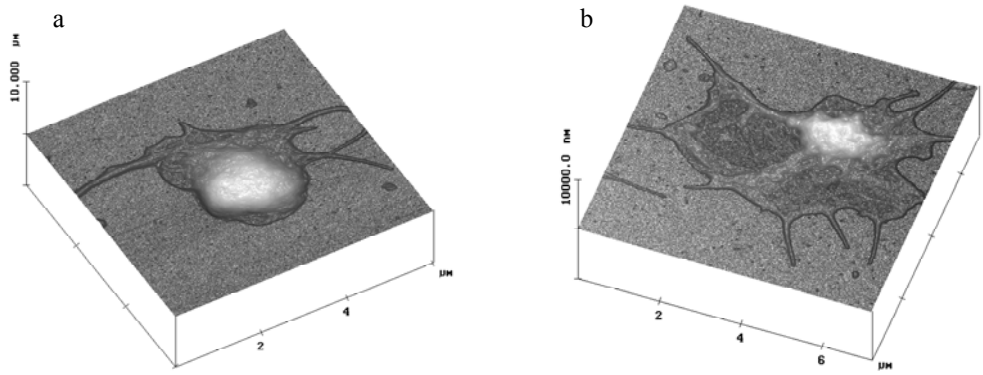

Fig. 3. SFM image of human platelets a – in the initial stage of the spreading process following mica adhesion; b – in an intermediate stage

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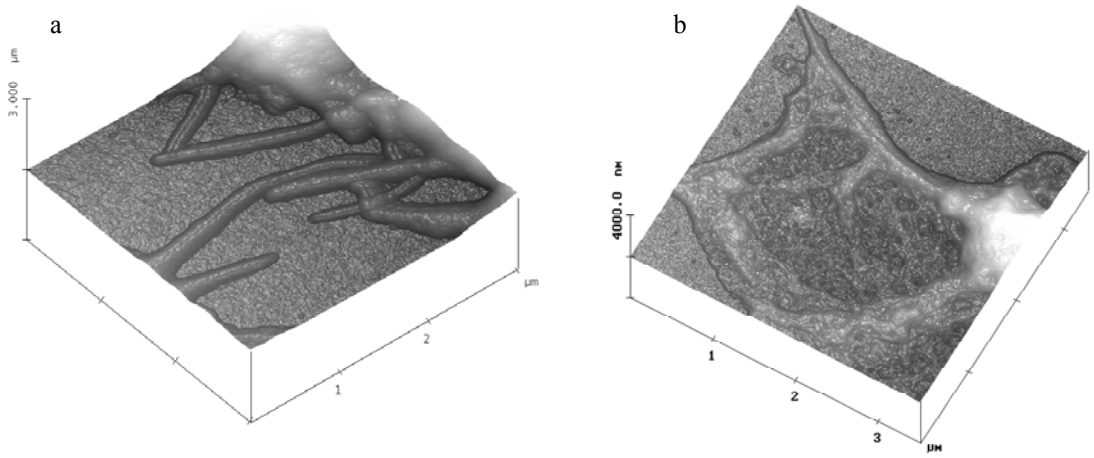

Fig. 4. SFM images of two different actin-based structures of mica-activated platelets: filopodia (a) and lamellipodia (b)

As the adhesion process advanced, the filopodia expanded laterally over the mica substrate, becoming lamellipodia (Fig. 4, b). Lamellipodia contain orthogonally arranged actin networks at platelet peripheries. The main lamellipodia function is to arrest vascular leakage by adhering to wounded surfaces. The lamellipodia thickness varied from 5 to 70 nm. Platelets in an intermediate stage of spreading had rounded shape with a few filopodia and lamelliapodia expanded between them. The cell width is ~6 µm and height is 400 nm (Fig. 3, b). SFM studies have demonstrated that in this stage of spreading process a significant number of granules move towards the front of the plasma membrane. Figure 5 clearly shows that some granules remain distributed over the lamellipodia. The release of granules left craters of variable size on the upper surface of the lamelliapodium.

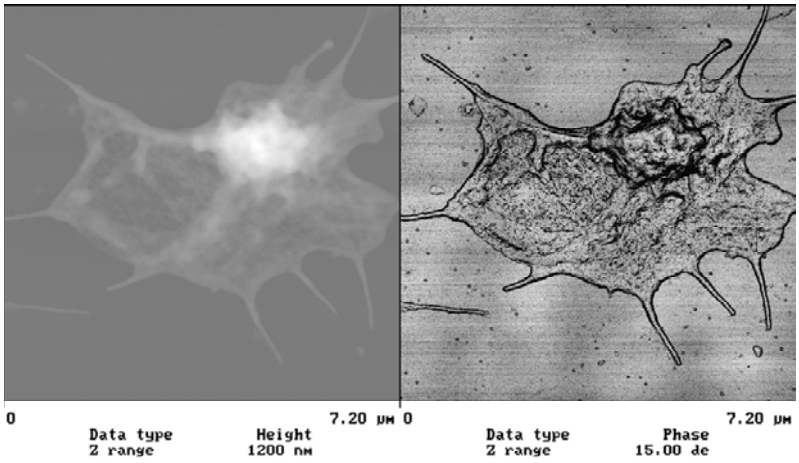

Fig. 5. SFM image of human platelet in an intermediate stage of the spreading process following mica adhesion

Morphological SFM investigations of mica surface-activated platelets have revealed that platelets spread laterally either symmetrically or asymmetrically with and

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without any filopodia appearance (Fig. 6, a, b). At the beginning of adhesion to mica platelets had rounded shape with filopodia projected over the substrate. Some filopodia may extend from 3 to 6 μm over the mica substrate before the lamellipodium begins to spread as a web connecting filopodia (Fig. 5). Several thick filopodia may extend and then stop. After that lamellipodium starts to spread laterally from one or more of filopodia (Fig. 6, b). Modification of filopodia to lamellipodia occurs when cytoskeleton changes from bundles of actin filaments in filopodia towards actin-myosin cortical contractile networks in lamellipodia [4].

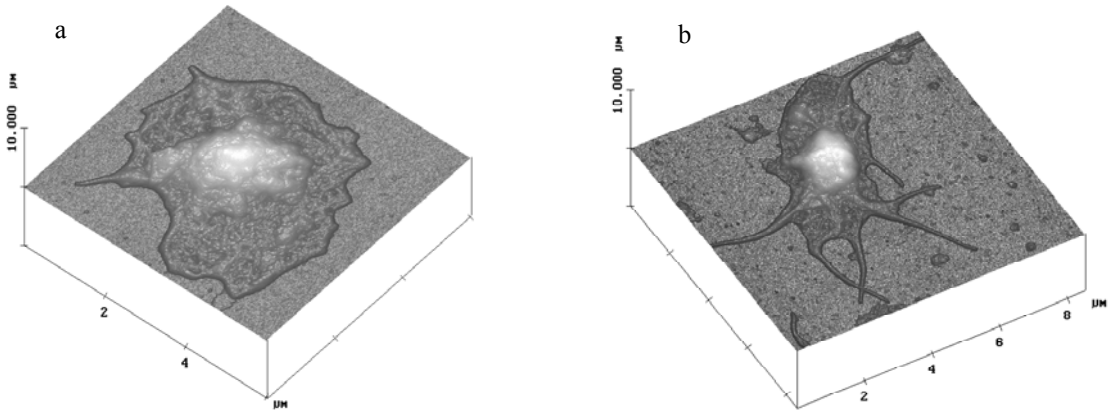

Fig. 6. SFM images of human platelet in an intermediate stage of the spreading process following mica adhesion: symmetrically (a) and asymmetrically (b) spreading

Figure 7 shows a platelet in fully spread stage. Platelets in the end of spreading process possessed a flattened round shape with a pseudonucleus containing many granules of different size. The diameter of granules varied from 250 to 400 nm.

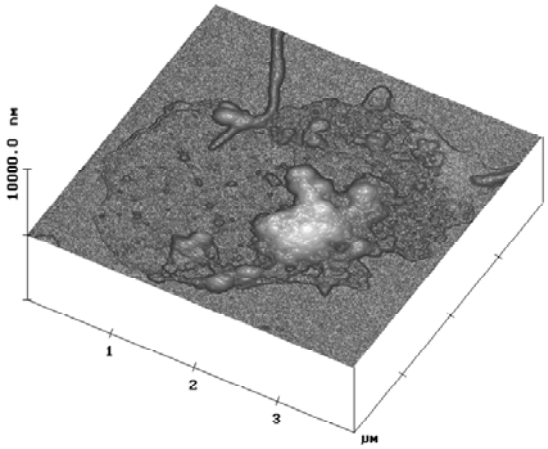

Fig. 7. SFM image of human platelet in a late stage of the spreading process following mica adhesion

Conclusions

In this study, we used SFM to demonstrate the surface morphology changes of mica-activated platelets. Investigations by SFM at high resolution have shown that

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discoid platelets undergo sequential stages of morphological changes after adhesion to mica: extension of short filopodia by round platelets; extension of long filopodia by round and partially spreading platelets; expansion of lamelliapodia between filopodia and granula motion; and finally, the fully spread form. SFM holds great promise as an instrument to visualize surface morphology changes occurring immediately after platelet activation in a various (air or liquid) environments with high spatial resolution on a submicron scale.

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