Fluorescent Microscopic Study of Endocytosis of Nanoparticles by Platelets

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We propose a method of precise measurement of the content of nanoparticles carrying a fluorescent label in platelets. Examination under a broadband filter does not allow distinguishing platelets containing and not containing nanoparticles despite different staining of platelets and nanoparticles. Print Screen image of the sample made at the moment of sample motion divides the colors of nanoparticles and platelets and yields two clear-cut spots of different colors on the monitor indicating the presence of nanoparticles in platelets.

Key Words: *platelets; nanoparticles; endocytosis; fluorescence*

Platelets can absorb various nano- and microparticles [3,4,6]. This capacity should be evaluated in each case of proven or presumptive penetration of these particles into the body. The information on the intensity of endocytosis is important, because removal of foreign micromaterial from the blood reflects the protective effect of platelets [1,2]. Another important aspect is evaluation of the effect of nanoparticles absorbed by platelets on their aggregation. Moreover, phagocytosis of nanoparticles affects the duration of their circulation in the blood. In view of small size of nanoparticles (below the resolving power of the light microscope) and platelets, the necessity of electron microscopy for evaluation of endocytosis becomes evident. This method can be used for the analysis of a great variety of nanoparticles; however, electron microscopy of some materials is very difficult, though possible. These are materials characterized by high hardness (metals, fullerenes, silicon) and therefore can hardly be ultratomized. Electron microscopy has some other limitations (high costs, laboriousness, and long procedure of specimen preparation). Here we propose an express method for reliable determination of nanoparticle endocytosis.

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MATERIALS AND METHODS

Platelet-rich plasma (PRP) was obtained from Chinchilla rabbits as described earlier [5]. In our experiments, 3.8% sodium citrate (1:9 v/v) was used as the anticoagulant. PRP was incubated with a suspension of nanoparticles of dry birch bark extract labeled with a green fluorochrome 3-methoxybenzatron (MBA; 60 min at 37°C). Aqueous suspension of nanoparticles (0.5 mg/ml) was added to PRP samples (1:10 v/v). After incubation, PRP smears were prepared on slides, stained with ethidium bromide (1:100,000 in PBS) for 10 min at 4°C, and fixed in 96% ethanol for 5-7 min. The preparations were analyzed under an Olympus BX51 fluorescent microscope equipped with filters with excitation/emission wavelength ratio of 545/605 nm (red band) and 495/519 nm (green band) using Cell F software.

RESULTS

Platelets containing mitochondrial DNA were well stained with DNA-specific fluorescent dye ethidium bromide. In the smear prepared before incubation they looked like small (~3 mm in diameter at ×1000) round orange formations (red filter). None internal structures were visualized. For detection of particles carrying green label, were examined platelets under the green filter.

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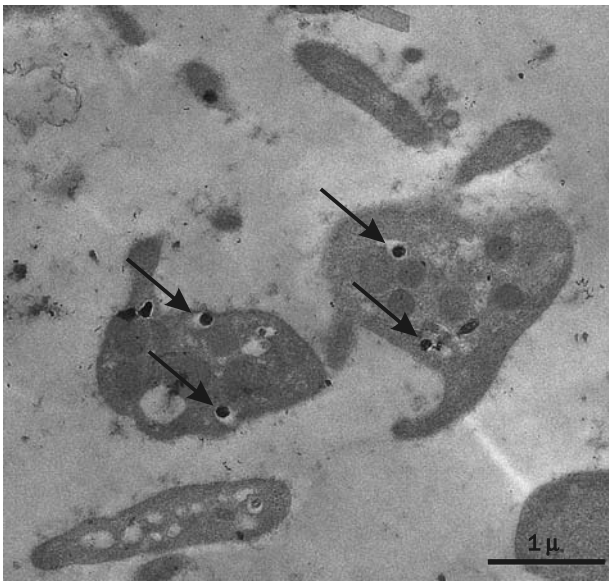

Fig. 1. Electron microscopy: platelets contain nanoparticles (arrows) phagocytozed during incubation.

Broadband filter allowed visualization of platelets under these illumination conditions. They just became more yellow. Epifluorescence microscopy with green filter showed that after incubation platelets became more yellow than before it. However, these changes were minor and the researcher observing them through the ocular could not be sure. No convincing proofs (images) could be obtained either. At the same time, parallel electron microscopy analysis showed that endocytosis of nanoparticles took place (Fig. 1) and was rather intensive, if we bear in bind that electron microscopy shows only small portion of nanoparticles captured by the platelet. Nevertheless, even this intensive phagocytosis was not clearly visualized under light microscope and fluorescent label did not simplify this task.

The problem was solved due to accidental observation. During rapid motion of the slide by the slide-moving device, the yellow-orange label from phagocytizing platelets separated into two similar by the shape and size spots of different colors: green and orange (Fig. 2 and 3). The difference was quite convincing and was easily seen on the monitor. For obtaining the image, it was sufficient to press Print Screen key on the keyboard during slide motion. Motion of platelets not incubated with nanoparticles was not accompanied by label separation (Fig. 2, *c*).

Thus, we develop a method of unerring detection of endocytosis of fluorescent-labeled nanoparticles by platelets, the smallest cells of the body. The proposed method has substantial limitations. It is suitable for nano- and microparticles purposefully introduced into the body and carrying a fluorescence label. The list

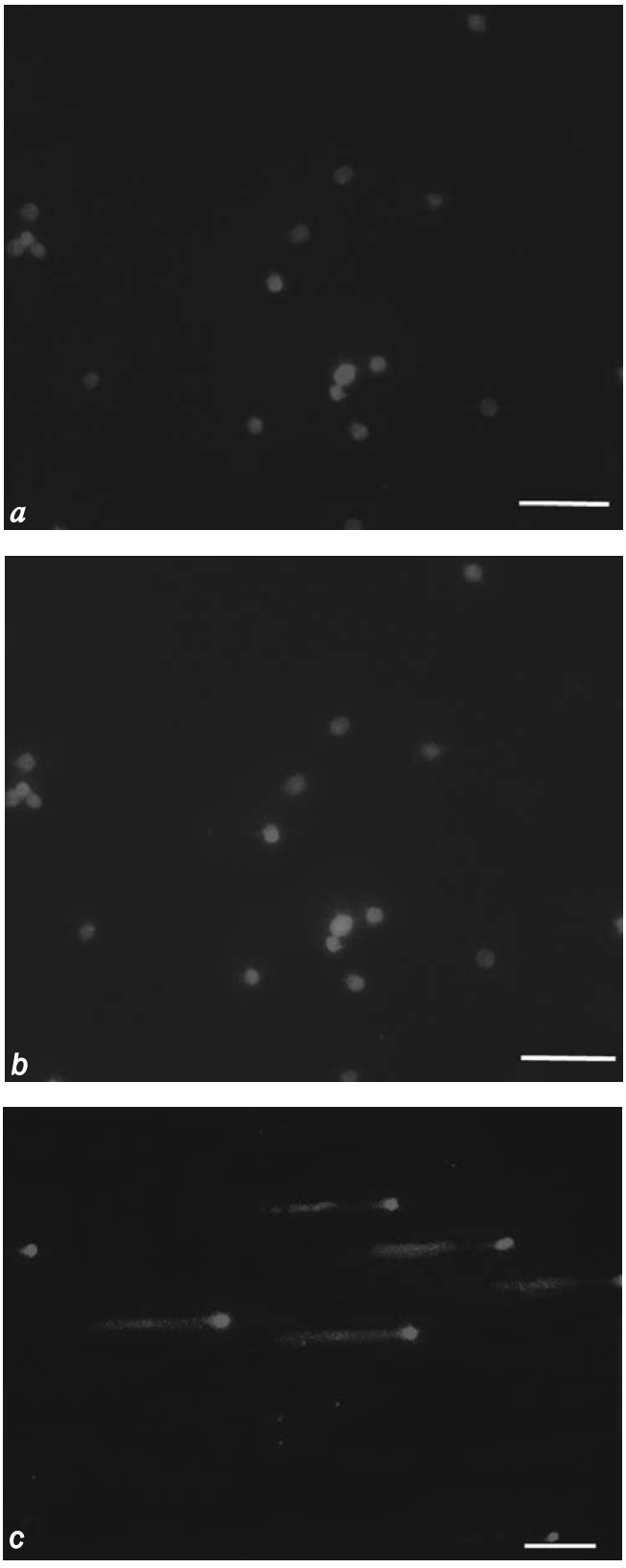

Fig. 2. PRP smear before incubation. Ethidium bromide staining Scale: 10 µ. *a*) red filter; *b*) green filter; *c*) green filter; Print Screen imaging during slide motion. blurred cell images without color separation.

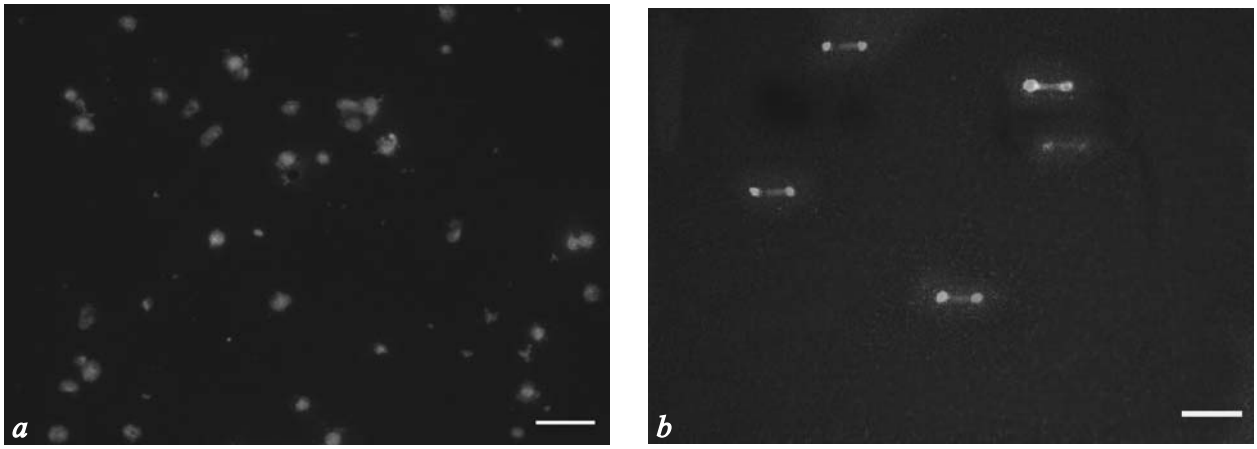

Fig. 3. PRP smear after incubation: fluorescent microscopy with green filter. Scale: 10 μ. *a*) motionless slide, platelets practically did not differ from cells before incubation; *b*) Print Screen imaging during slide motion: each platelet is presented by two clearly separated color spots (green and yellow).

of these particles increases day by day, therefore our method can be needed in nanomedicine, a promising and rapidly developing area of science.

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