Žalių fluorescencinių grafeno kvantinių taškų sąveika su žmogaus trombocitais

Interaction of green fluorescent graphene quantum dots with human platelets

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Green fluorescent graphene quantum dots (GQDs) are promising nano-agents for optically-guided targeted drug delivery. Thrombus formation in response to the action of nanomaterials [1] is one of the significant obstacles in biomedical applications, therefore, the establishment of the aggregation activity of nanoparticles and the mechanisms of their interaction with platelets and blood plasma proteins is a primary task in the development of nanoparticle-based treatment and diagnosis. Thus, the main goal of the study was to reveal the accumulation of GQDs in platelets, investigate the activation/aggregation of platelets in the presence of GQDs, and demonstrate the role of the blood plasma proteins in the GQD aggregation properties. Platelet-rich plasma (PRP) was used to evaluate the impact of plasma proteins.

Aggregation activity of GQDs at a final concentration of 50 μg/mL towards platelets and platelets in PRP was investigated by fluorescence microscopy. Fluorescence images of cells were analyzed using a custom Matlab R2009b (MathWorks, USA) script.

It was revealed that GQDs induce the activation of platelets. Cells without GQDs were visualized via autofluorescence when excited with λ_{ex}=473 nm (Fig. 1a, g) and fluorescence intensity analysis was performed (Fig.1c, i). The fluorescence of GQDs accumulated in platelets (Fig.1l) significantly exceeded the autofluorescence of cells in control samples. However, slight fluorescence intensity increase was also observed for GQD-treated platelets in PRP (Fig.1f). It was assumed that some accumulation of GQDs in activated cells in PRP was also present. Platelets and cells in PRP non-treated with GQDs had round shape without any features of activation (Fig.1a and g), with similar size distribution (Fig.1b and h), while in GQD-treated cells significant morphological changes were observed (Fig.1d and j). Cell size analysis (Fig.1b, e, h, and k) revealed an increase in platelet size for GQD-treated platelets and cells in PRP. A huge fraction of platelet aggregates with sizes exceeding the average size of a single platelet by more than 20 times was detected in samples with GQD-treated cells (agglomerates are marked with arrows, Fig.1j). In PRP platelets were present as single cells, however, a significant change in their shape was also observed (Fig.1d) as compared with control cells (Fig.1g).

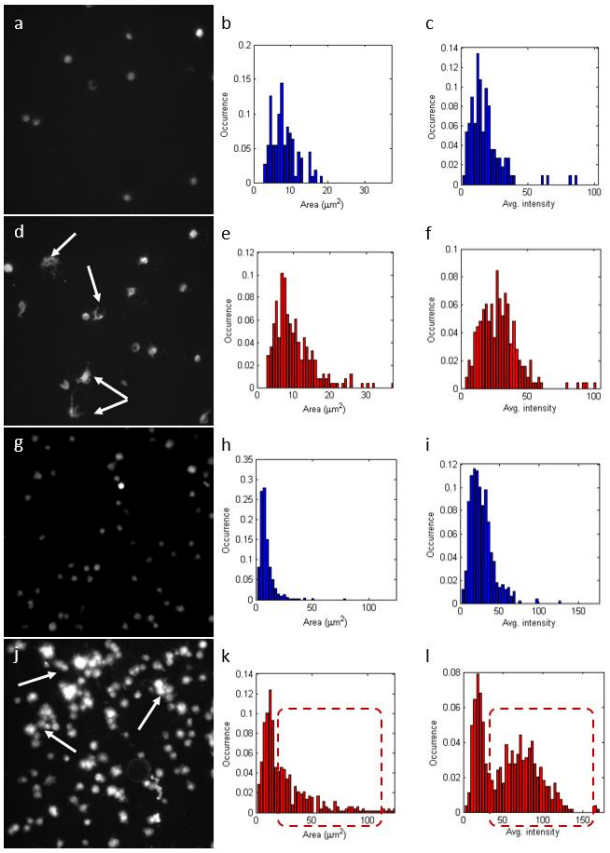

Fig.1. Fluorescence microscopy analysis of platelets in PRP (a-c) and isolated platelets (g-i): (b, e, h, k) – platelet area distribution; (c, f, i, l) – distribution of average intensity per cell; (a-c, g-i) – non-treated cells; (d-f, j-l) – GQD-treated cells.

Summing up, GQDs are accumulated in platelets, lead to platelet activation and aggregation at the concentration of 50 μg/mL. The presence of blood plasma proteins in the medium (PRP model) prevents platelet aggregation due to the formation of so-called bio-corona around GQDs but does not hide the activation potential of GQDs.

Keywords: fluorescence microscopy, platelets, graphene quantum dots, platelet-rich plasma.

Literature

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