GRAPHENE QUANTUM DOTS AS PROMISING NANOSTRUCTURES FOR STUDYING REDOX PROCESSES IN NEUTROPHILS

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Graphene quantum dots (GQDs) belong to the group of promising nanomaterials for the development of bio-sensing systems. GQDs consist of several sp2-hybridized graphene sheets, with a lateral size from several to 10 nm. The GQDs edge can be functionalized with hydroxyl, carbonyl, carboxyl groups. Functionalized GQDs are water-soluble and more stable in physiological media than graphene oxide. GQDs can also act as a donor/acceptor of electrons, thus being sensitive to reduction/oxidation processes in living systems, e.g., neutrophils.

The aim of this work was to investigate the mechanisms of interaction of GQDs with human neutrophils and reveal the potential for the detection of reactive oxygen and chlorine species (ROS/RCS) during cell activation.

Neutrophils were isolated from the whole blood of healthy donors. GQDs were added to the cells at a concentration of 50 μg/mL, the incubation time was 30-90 min. Intracellular GQDs distribution, F-actin reorganization, myeloperoxidase (MPO) redistribution and release were performed via fluorescence microscopy.

Influence of GQDs on the cytoskeleton and myeloperoxidase release in neutrophiles

F-Actin distribution in neutrophiles under GQDs treatment

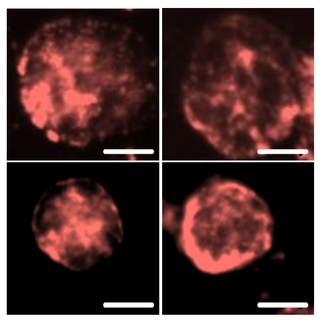
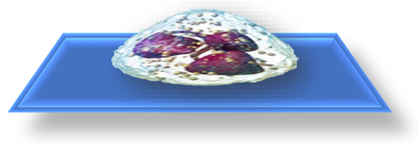


Figure 1. F-Actin distribution in control neutrophiles. Scale bar 15 μm. F-Actin stained with AlexaFluor Phalloidine-532. Excitation 532nm, emission maximum 620 nm. Adhesion time 30 minutes.

Stimulated by adhesion to Si wafer



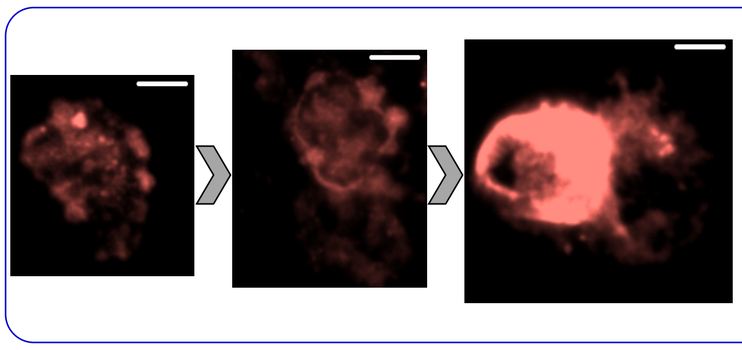


Figure 2. F-Actin distribution in neutrophiles pretreated with GQDs for 30 minutes. Scale bar 15 μm. F-Actin stained with AlexaFluor Phalloidine-532. Excitation 532nm, emission maximum 620 nm. Adhesion time 30 minutes. GQDs concentration 50 μg/mL

MPO distribution in neutrophiles under GQDs treatment

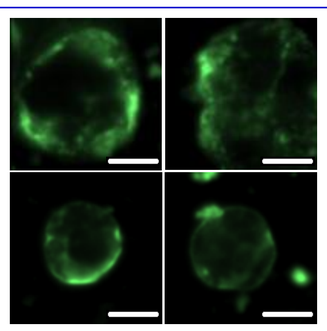
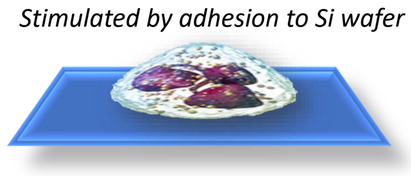


Figure 3. MPO distribution in control neutrophiles. Scale bar 15 μm. MPO stained with FITC. Excitation 470 nm, emission maximum 530 nm. Adhesion time 30 minutes.

Stimulated by adhesion to Si wafer



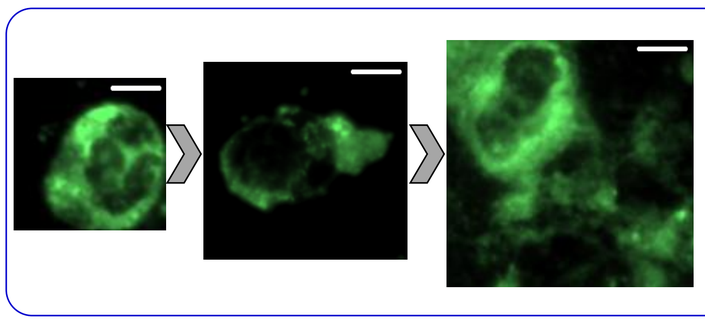
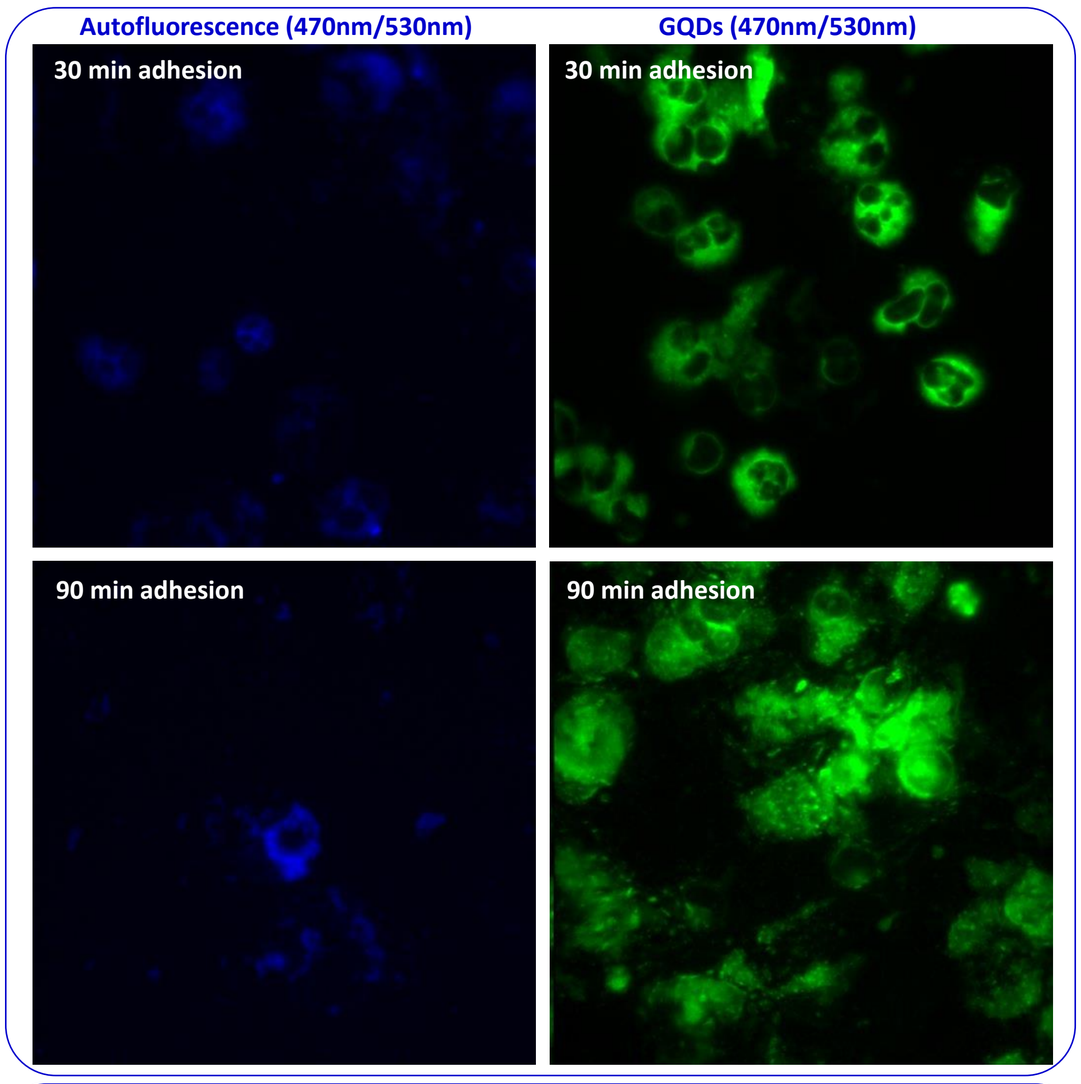


Figure 4. MPO distribution in neutrophiles pretreated with GQDs for 30 minutes. Scale bar 15 μm. MPO stained with FITC. Excitation 470 nm, emission maximum 530 nm. Adhesion time 30 minutes. GQDs concentration 50 μg/mL

Incubation of neutrophils with GQDs for 30 minutes led to their accumulation in cells and cell activation, although no cytotoxic effect was revealed. A more pronounced activation of neutrophils exposed to GQDs for 90 minutes was observed. This was manifested in a greater spreading of cells than in control, in the modification of actin cytoskeleton (Fig. 1 and 2), secretion of MPO to the outer space and the formation of neutrophil extracellular trap-like structures (Fig. 3 and 4). The intensity of GQDs fluorescence in neutrophils after 30 min of cell adhesion was three times higher than after 90 min of adhesion, indicating the destruction and/or disruption of the GQDs structure after penetration into neutrophils (Fig. 5). Hypochlorite ions, produced via neutrophils activation and MPO release led to a decrease in the GQD fluorescence via their structure changes allowing to estimate the intensity of reduction/oxidation processes (RCS increase) (Fig. 6).

Accumulation of GQDs in neutrophiles (Figure 5)



Mechanism of HClO sensing by GQDs in neutrophils

In an aqueous solution, NaClO dissociates and hydrolyzes to give hydroxide ions:

$$NaClO \rightarrow Na^+ + ClO^-, \qquad ClO^- + H_2O \rightleftharpoons HClO + OH^-$$

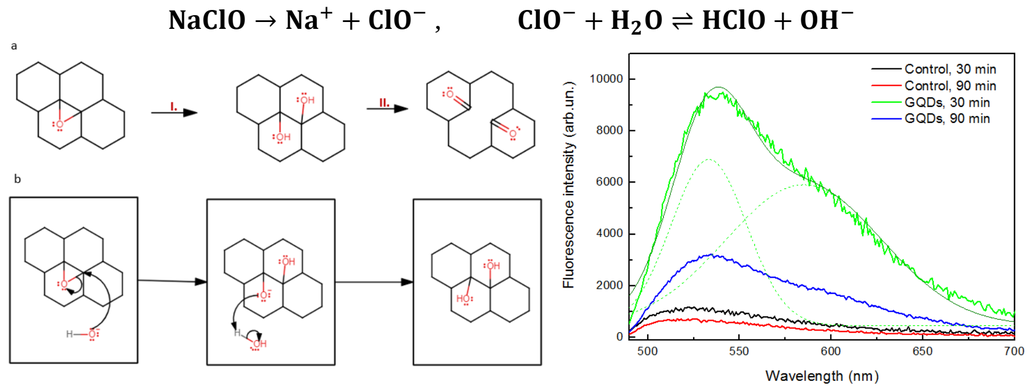
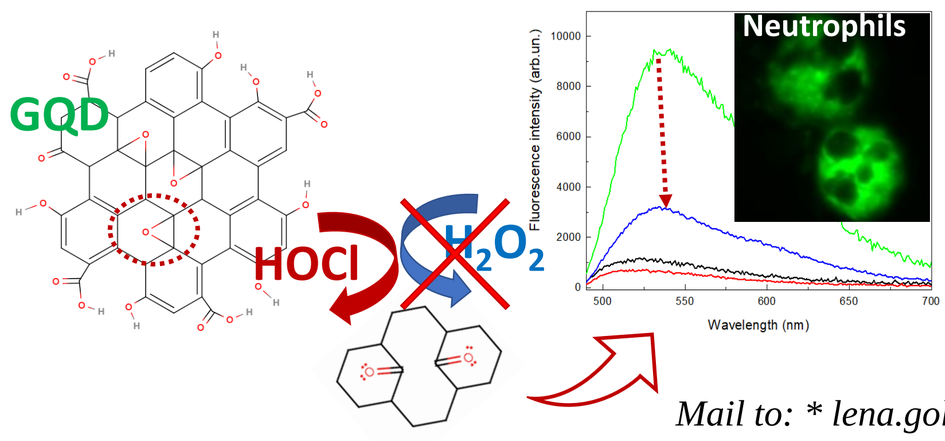


Figure 6. Fluorescence spectra of **neutrophiles** in control samples (black and red) and pretreated with GQDs (green and blue) for 30 and 90 minutes respectively. Spectra are averaged spectra from 5 different points on the sample. Excitation wavelength 470 nm.

Conclusions

- ✓ GQDs are accumulated in human neutrophils.
- ✓ GQDs serve as a selective fluorescent sensor of hypochlorous acid in biosystems.
- ✓ HClO opens epoxide ring in GQD, damaging carbonic core by excessive C=O formation
- ✓ GQDs induce actin reorganization in neutrophils, MPO release and NET-osis.
- ✓ **GQDs biodegrade in human neutrophils via MPO-produced hypochlorous acid.**



- ▫ **HOCl sensitive**
- ▫ **Biodegradable**
- ▫ **Suitable for cell imaging**

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