DO NANOPLASTICS CHANGE RED BLOOD CELL VISCOELASTICITY? A PILOT STUDY HARNESSING QUARTZ CRYSTAL MICROBALANCE WITH DISSIPATION MONITORING

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BACKGROUND

On grounds of versatility and affordability, plastic materials permeate the majority of current human activities. Since the 1950s, plastic demand and manufacturing grew exponentially together with concurrent waste dumping. It is estimated that by 2050 roughly 12 thousand millions tons of plastic waste will be in landfills or in natural environment [1]. Nanoplastics are plastic particles smaller than 1000 nm in size [2]. Most NPs are unintentionally produced during wear and tear and/or environmental degradation of larger plastic stuffs (incidental or secondary NP**s**), while the least part is manufactured for industrial uses (primary NPs). Hence, NPs are fast-growing, ubiquitous pollutants posing increasing worldwide concern about the potential adverse effects to human and environmental health. Whatever the source and human route of exposure (ingestion, dermal absorption, inhalation), NPs can cross biological barriers, enter the bloodstream and interact with the red blood cells (RBCs), the most abundant circulating cells, thereby possibly impairing their properties and functions. RBCs exhibit exceptionally high deformability which lets them squeeze in the thinnest capillaries to deliver oxygen to tissues. RBC deformability depends on cell viscoelastic properties. Simulation studies showed that nanosized polystyrene particles can disrupt cell membrane structure and lateral organization [3], an effect arguably leading to viscoelasticity (VE) changes.

AIM

The present study was undertaken to investigate NP effect on human RBC viscoelasticity by harnessing a novel protocol based on the label-free, minimally invasive Quartz Crystal Microbalance with dissipation monitoring (QCM-D) technology. Such technology exploits high frequency acoustic waves to probe the viscoelastic properties of thin films in contact with the crystal surface and is adequate for investigating the viscoelasticity of liquid-bathed soft materials, such as adherent cells are [4].

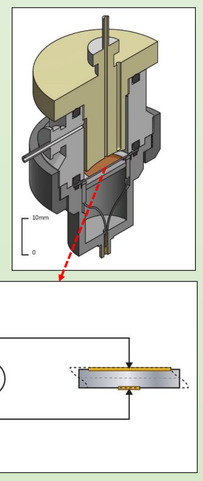
MATERIALS & METHODS

THE QCM-D APPARATUS

The apparatus for VE analysis consisted of i) a commercial QCM sensor, ii) a QCM holder designed and built in our workshop, made of a lower part accommodating the crystal sensor and the electrical contacts, and an upper part serving as sample chamber (Fig. 1), iii) a thermostat controller, and iv) a computer-operated vector network analyzer for impedance analysis.

Changes of the fundamental resonance frequency (Δf) and bandwidth (ΔΓ) were continuously recorded and ΔΓ was adopted as a measure of VE.

Figure 1



NP SUSPENSIONS

100 nm and 50 nm nominal diameter polystyrene NPs (NP100 and NP50, respectively) and 25 nm nominal diameter polymethacrylate NPs (NP25) were obtained from Micromod Partikeltechnologie GmbH (Rostok, Germany).

NP dispersion state in either water or working solution (Hepes-buffered saline solution, HBS) were characterized by Dynamic Light Scattering (DLS, Zetasizer Nano ZS, Malvern, Worcestershire, UK).

RBC PREPARATION

A freely emerging drop of capillary blood was obtained from healthy donor by finger pricking and immediately dispersed in HBS. RBC were isolated by three low-speed centrifugation**s** and resuspensions in HBS and used within 1 hour.

QCM-D MEASUREMENTS

To ensure stable cell adhesion to the sensor surface throughout the readings, a glutaraldehyde-modified, cysteamine self-assembled monolayer (SAM) was cast onto the gold electrode surface. After a stable signal was reached in air, HBS was added into the QCM chamber and the signal in liquid acquired for just a few minutes. HBS was then replaced by the RBC suspension. After signal stabilization, unbound RBCs were gently sucked-off, fresh HBS was poured, and the RBC signal was acquired for about 10 min before cell challenging with NPs (1, 10 or 100 µg/ml). RBCs exposed to stressors known to alter cell viscoelasticy served as positive controls.

RBC MORPHOLOGY

After cell fixation (0.05% glutaraldehyde,4h, RT) and air-drying, morphology of QCM-adherent RBCs was assessed by both reflected light microscopy and atomic force microscopy (MultiView1000, Nanonics Imaging Ltd, Jerusalem, Israel).

RESULTS AND DISCUSSION

NP CHARACTERIZATION

Table 1 reports the NP hydrodynamic diameter (Z-average) and polydispersity index (PDI). All particles were in the nanosize range. However, while the Z-avg of NP100 and NP50 samples were in line with the expected size, the Z-avg of 25nm NPs was slightly higher both in water and HBS likely accountable for the presence of some NP agglomerates. Suspensions in HBS were stable up to at least 2 h from the preparation.

Table 1

NP (100 µg/mL)	H_2O – 0h		HBS – 0h		HBS - 2h	
	Z-average	PDI	Z-average	PDI	Z-average	PDI
NP 100	115.1 ± 1.1	0.006	111.6 ± 0.4	0.014	110.9 ± 1.4	0.014
NP 50	54.4 ± 0.4	0.026	52.8 ± 0.5	0.047	58.2 ± 0.8	0.086
NP25	40.1 ± 0.3	0.135	39.1 ± 0.1	0.080	39.1 ± 0.1	0.086

QCM-D RESPONSE TO RBC ADHESION

As expected, cell sedimentation produced a progressive increase of both ΔΓ and Δf up to reach a steady-state, standing for maximal RBC adhesion (Fig. 2a). Adherent RBCs were homogeneously distributed over the sensor surface and showed a normal discocytic shape (Fig.2b). The increase of the ΔΓ to -Δf ratio as a function of the surface coverage (represented by –Δf in Fig. 2c) shows that adhered RBCs behave as a homogeneous film [5]. Hence, ΔΓ was confirmed to serve as a trustworthy measure of the RBC viscoelasticity.

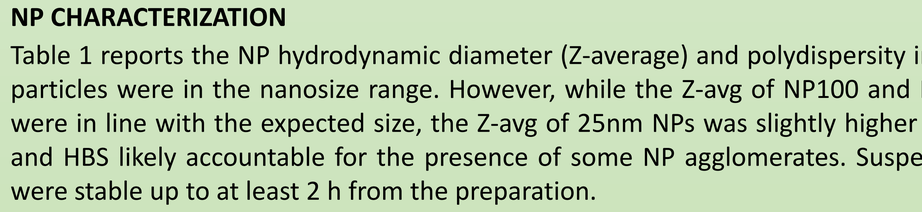


Figure 2

RBC VISCOELASTICITY CHANGES IN RESPONSE TO RECOGNIZED STRESSORS

RBC ΔΓ was significantly changed by different physical and chemical stressors known to alter cell viscosity (temperature, Fig. 3a, and medium osmolarity, Fig. 3b), or cell viscoelasticity (glutaraldehyde, a non-specific cross-linking agent, Fig. 3c, and methyl-ß-cyclodextrin, MßCD, a drug able to change membrane stiffness by changing the membrane cholesterol content, Fig.3d).

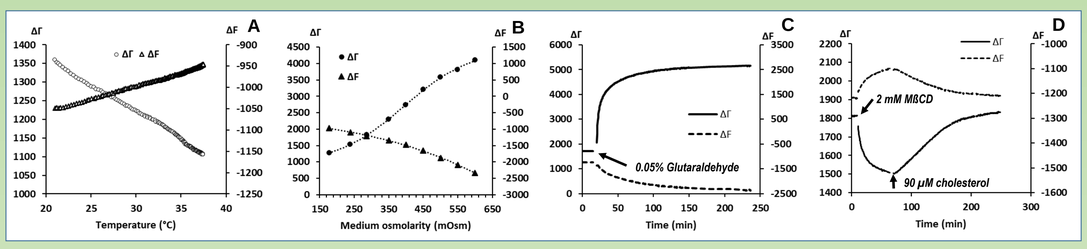
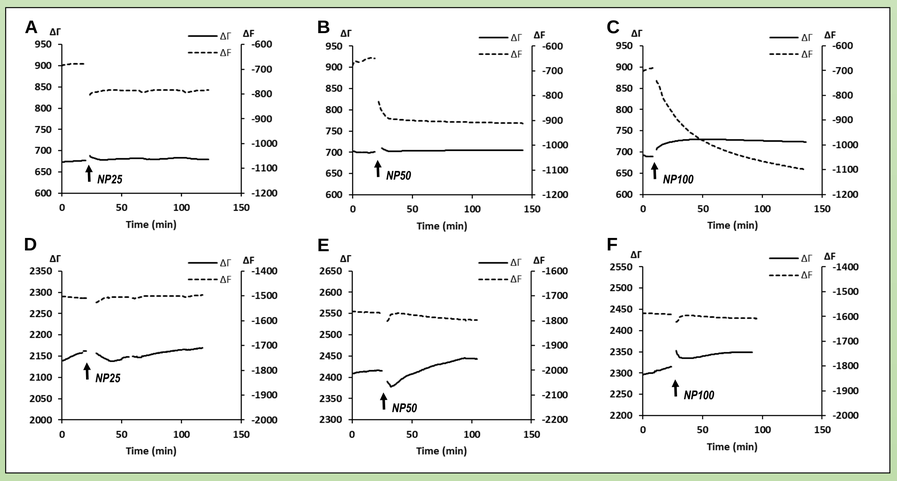


Figure 3

EFFECT OF NPs ON RBC VISCOELASTICITY

In the absence of cells, 100 µg/ml NPs induced a size-dependent increase of the Δf modulus (Fig. 4a, b, c) suggesting a weak interaction of the particles with the sensor surface. As expected for rigid materials, ΔΓ was << Δf, the only significant effect being produced by the largest particles. In the presence of the RBC monolayer, the NP effect on Δf was abolished, likely due to the drastic reduction of the direct particles-sensor interaction. Notably, regardless of the particle size, ΔΓ showed almost irrelevant changes of RBC VE (Fig. 4d, e, f). Similar results were obtained at lower NP concentrations (not shown).

Figure 4



EFFECT OF NPs ON RBC MORPHOLOGY

NPs altered the morphology of adhered RBCs by inducing echinocytosis in a size- and dose-dependent manner, the smallest ones being the more effective. Figure 5 shows two representative AFM images of control (A) and treated cells (B, 100 µg/ml NP25).

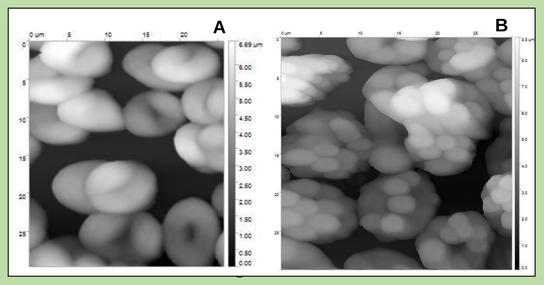


Figure 5

CONCLUSIONS

Our results show that:

- ✓ The QCM-D-based protocol presented here is a convenient, powerful tool to assess RBC viscoelasticity changes induced by different stressors
- ✓ In the dose range tested, the interaction of 25 to 100 nm NPs with RBCs did not seemingly alter RBC viscoelasticity albeit a significant alteration of cell morphology was produced

Further studies are under way to bolster and expand our preliminary findings by investigating the effects of NPs exposing different surficial charges.

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