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Pursuing Assessment of Extracellular Particles in Diluted Blood by Interferometric Light Microscopy and UV-vis Spectrophotometry

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Methods

Study included 3 samples from human blood, Blood was taken into an evacuated tube with trisodium citrate anticoagulant and processed fresh. Blood was diluted with physiological saline solution. Plasma was obtained by centrifugation of blood and diluted with physiological solution. Diluted blood was centrifuged to sediment erythrocytes and larger segments. Number density and hydrodynamic diameter of Eps were assessed by Videodrop (Myriadelab, Paris, France) interferometric light microscope. Absorption of UV and visual light at 260 and 280 nm was assessed by the Nanodrop spectrophotometer (Thermofischer Scientific, Waltham, MA, USA). The absorbance ratio A280/A260 indicates the protein/nucleic acid content of the sample.

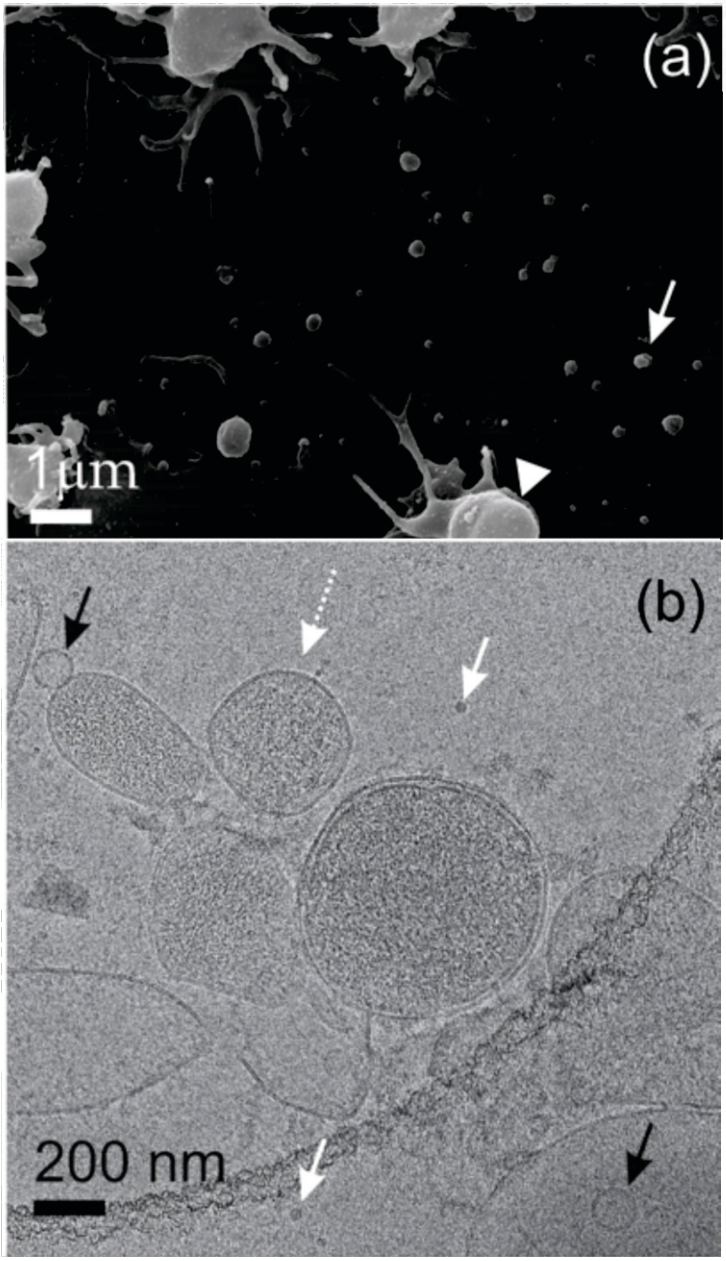

Figure 1. (a): EPs in canine plasma as observed by the Scanning Electron Microscope; white triangle points to a platelet, white arrow points to an EP; (b): EPs as observed by the Cryogenic Transmission Electron Microscope; dashed white arrow points to a large vesicle, black arrows point to smaller vesicles, white arrows point to molecular complexes. From Korenjak B, et al., Cells, 2024; 13(24):2054. https://doi.org/10.3390/cells13242054

Outline

Harvesting of EPs from body fluids and their assessment is challenging as processing significantly affects the samples. A recently developed technique Interferometric Light Microscopy (ILM) enabled determination of the number density n and the hydrodynamic diameter D_h of EPs (sized 80 nm to 500 nm) in samples which may contain larger particles (cells) and smaller particles (lipoproteins). In order to produce an observable signal, a threshold saturation of light is reached by appropriate dilution of samples with physiological solution thereby **avoiding isolation** of EPs prior to assessment. Figure 3 shows n, D_h and A280/A260 in dependence on the centripetal acceleration of the centrifuge rotor Xg, where g is the gravity acceleration of the Earth, and on the dilution factor R.
It is indicated that centrifugation up to cca 1000g does not affect n, D_h or A280/A260. In contrast, dilution is important for n and D_h. Too strong dilution induces aggregation of particles resulting in lower n and higher D_h (Figure 4). We estimated the optimal dilution of blood 10x and optimal settings of the centrifuge: room temperature, time 10 minutes and centripetal acceleration of the centrifuge rotor 500g.

Results

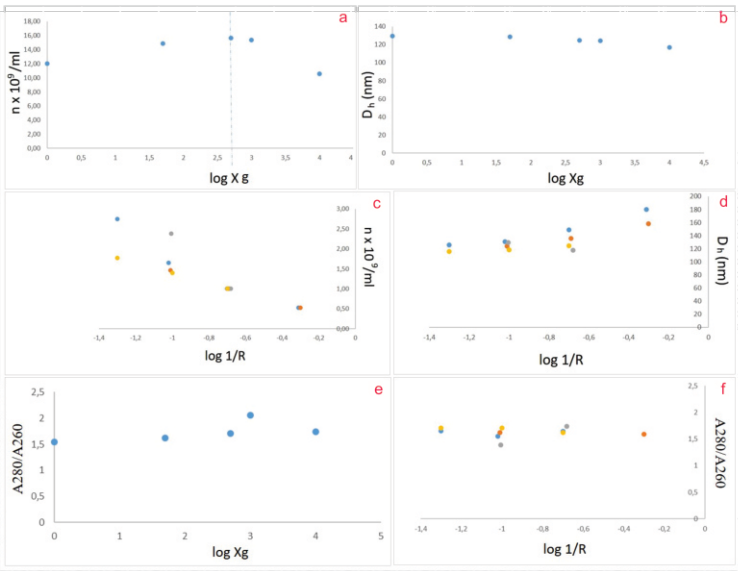

Figure 2. (a): Number density of EPs in 10x diluted blood in dependence on the centripetal acceleration of the centrifuge rotor Xg; (b): hydrodynamic diameter of EPs D_h in dependence on the centripetal acceleration of the centrifuge rotor Xg; (c): number density of EPs in dependence on the dilution of blood 1/R; (d): hydrodynamic diameter of EPs D_h in dependence on the dilution of blood 1/R; (e): absorbance ratio A280/A260 in dependence on the centripetal acceleration of the centrifuge rotor Xg; (f): absorbance ratio A280/A260 in dependence on the centripetal acceleration of the dilution of blood 1/R.

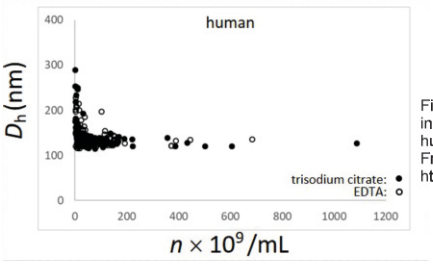

Figure 3. (a): Hydrodynamic diameter of EPs D_h in dependence on the number density of EPs in human plasma exhibiting the swarm effect. From (Korenjak B, et al., Cells, 2024, 13, 2054. https://doi.org/10.3390/cells13242054)

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