Long-term *in vitro* exposure to low-level perfluorooctanoic acid impairs human vascular endothelial cell function

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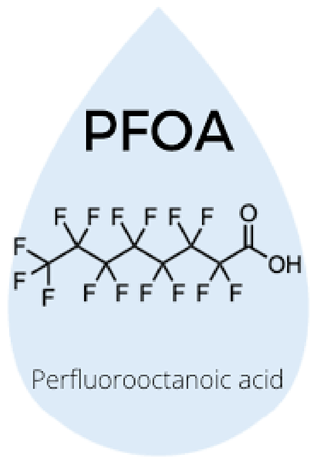
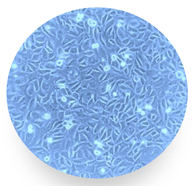
INTRODUCTION

Perfluorooctanoic acid (PFOA), a persistent environmental contaminant often termed a „forever chemical“, is commonly used in various industrial and consumer products. PFOA is recognized for its toxic effects on the nervous, endocrine, and reproductive systems, while emerging research suggests it may also impair vascular function, potentially contributing to cardiovascular diseases. However, the impact of PFOA on endothelial cell damage and endothelial dysfunction remains unclear. In particular, there is a lack of *in vitro* studies that mimic realistic exposure scenarios.

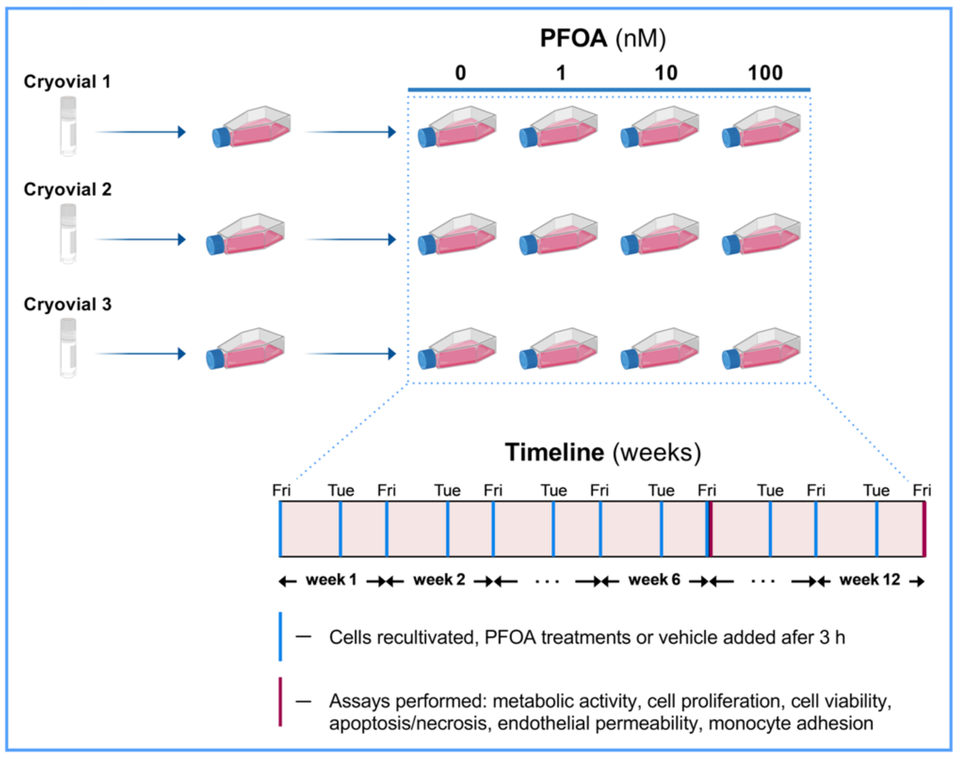


The goal of this study was to investigate the effects of long-term low-level exposure to PFOA on cell proliferation, survival, endothelial permeability, and monocyte adhesion to human endothelial cells *in vitro*.

METHODS



EA.hy926 cells → Grown at 37 °C in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, 1.5 g/L $NaHCO_3$, 0.11 g/L sodium pyruvate, 10 mM HEPES, and 4% HAT (5 mM sodium hypoxanthine, 20 µM aminopterin, 0.8 mM thymidine).



Metabolic activity → alamarBlue™ assay

Cell viability → Trypan blue dye-exclusion test

Cell proliferation → Cell counting (Luna II automated cell counter)

Endothelial permeability → Two-compartment permeability assay (Transwell®) with fluorescently labeled dextran (Mw 40 kDa)

Apoptosis and necrosis → Annexin V-FITC and propidium iodide via flow cytometry (Amnis® ImageStream®X Mk II Imaging Flow Cytometer, Luminex Corporation, Austin, TX, USA)

Monocyte adhesion → Calcein AM-labeled U937 human monocytic cells, fluorescence microscope

CONCLUSIONS

- No significant effect on metabolic activity and cell viability at any concentration and time point; however, after 12 weeks, reduced cell proliferation at 100 nM PFOA
- A slight decrease in live cells and an increase in necrotic cells after 6 weeks of exposure to 1 nM PFOA; more pronounced at all concentrations after 12 weeks
- A dose-dependent increase in endothelial permeability after 12 weeks
- Monocyte adhesion to endothelial cells significantly elevated after 6 and 12 weeks of exposure to 10 nM and 100 nM PFOA, respectively

In summary → the data support the hypothesis that exposure to PFOA may contribute to endothelial dysfunction.

RESULTS

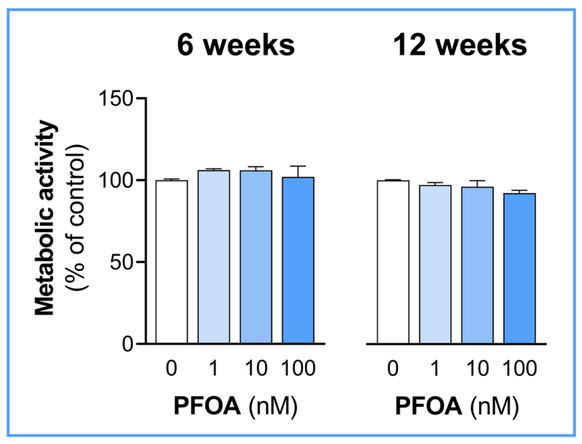


Figure 1. The effect of long-term exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA on metabolic activity assessed by the alamarBlue™ assay. Results were expressed relative to the vehicle-treated control (0 nM PFOA; 100%).

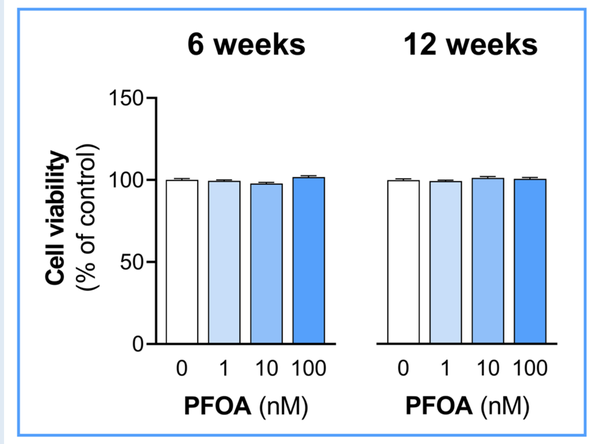


Figure 2. The effect of long-term exposure of EA.hy 926 cells to 1 nM, 10 nM, and 100 nM PFOA on cell viability assessed by the Trypan blue dye-exclusion test. Results were expressed relative to the vehicle-treated control (0 nM PFOA; 100%).

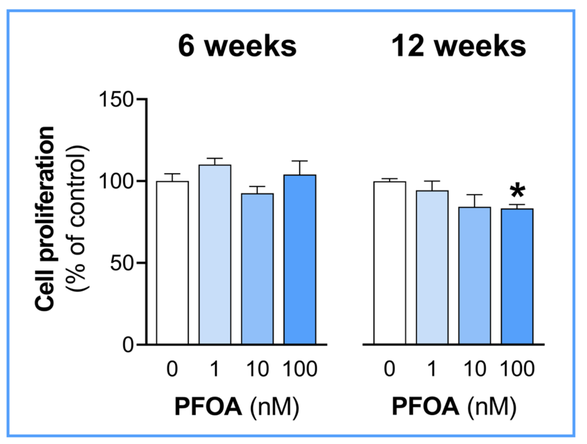


Figure 3. The effect of long-term exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA on cell proliferation assessed by Luna II automated cell counter. Results were expressed relative to the vehicle-treated control (0 nM PFOA; 100%).

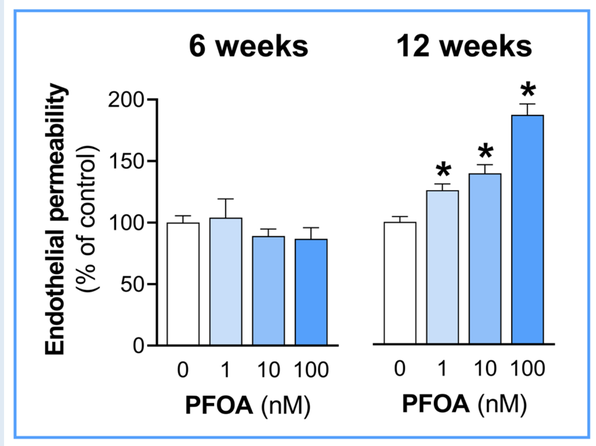


Figure 4. The effect of long-term exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA on endothelial permeability assessed by the Transwell® assay with fluorescently labeled dextran. Results were expressed relative to the vehicle-treated control (0 nM PFOA; 100%).

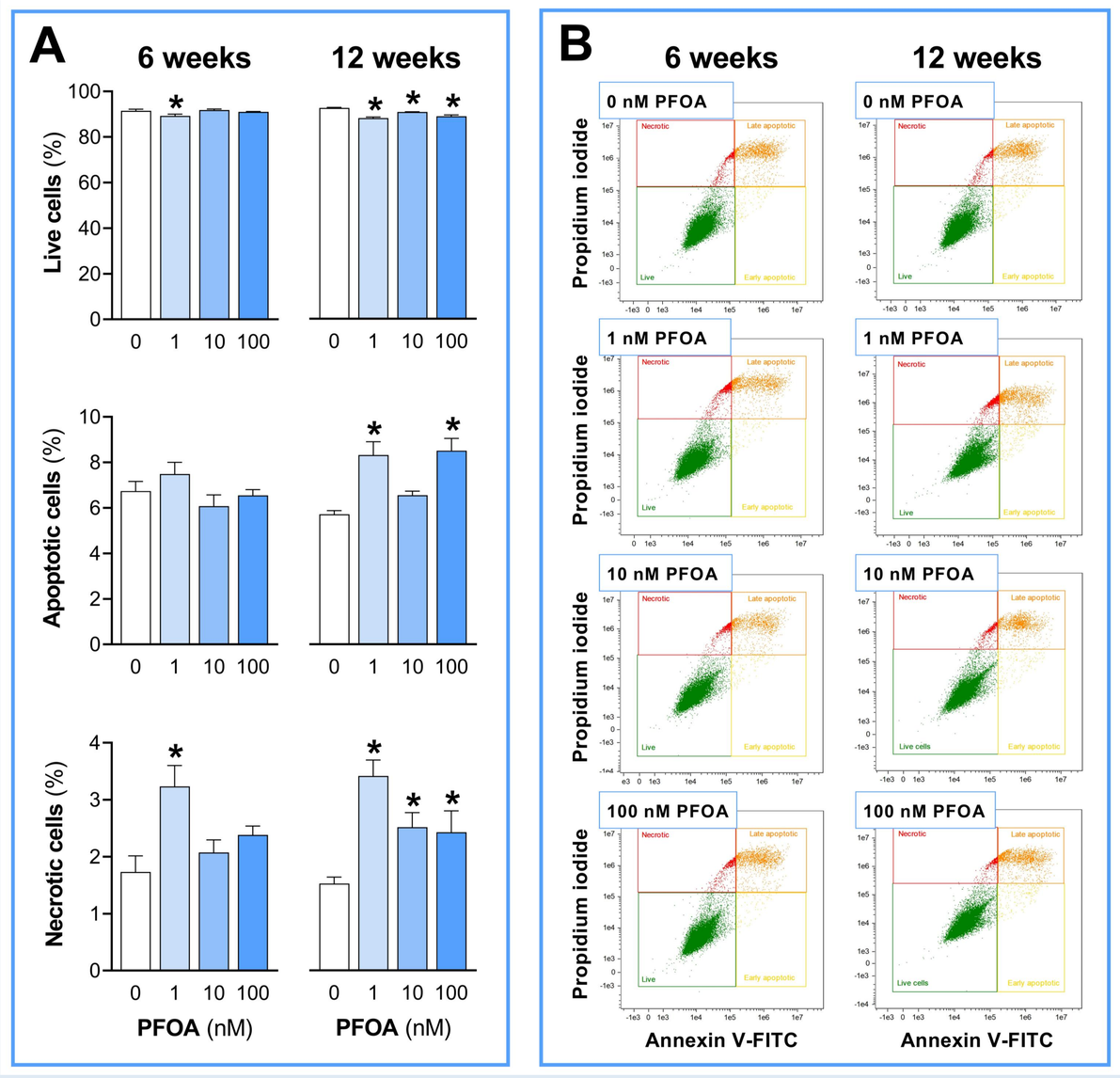


Figure 5. The effect of long-term exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA on apoptosis and necrosis assessed by the annexin V-FITC and propidium iodide fluorescence on a flow cytometer. (A) Results were expressed as the percentage of live, apoptotic, and necrotic cells. (B) Representative dot-plots from flow cytometry.

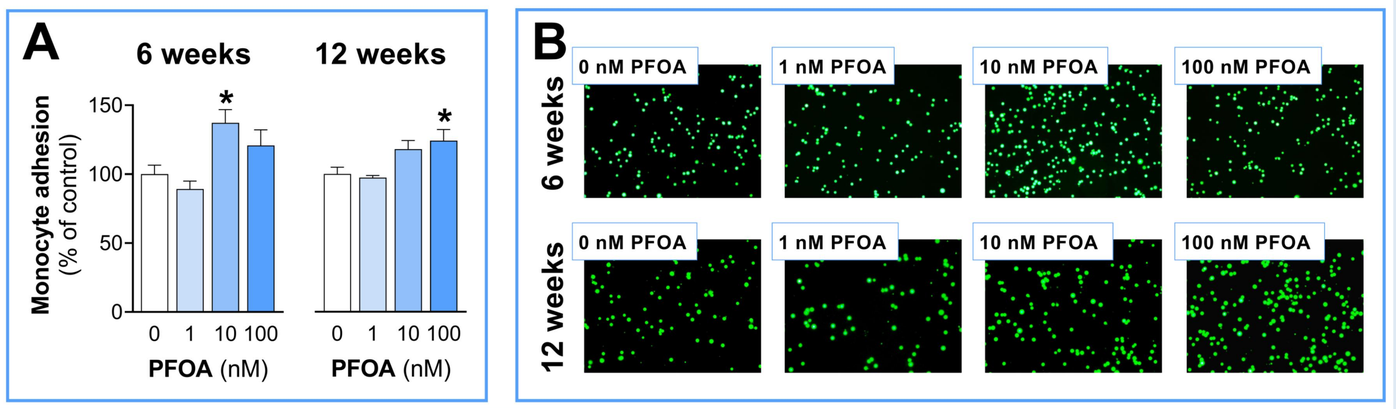


Figure 6. The effect of long-term exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA on monocyte adhesion assessed by the binding of calcein AM-labeled U937 human monocytic cells. (A) Results were expressed relative to the vehicle-treated control (0 nM PFOA; 100%). (B) Representative fluorescent photomicrographs.

Acknowledgements

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