Long-term low-level exposure to perfluorooctanoic acid affects the survival of human endothelial cells *in vitro*

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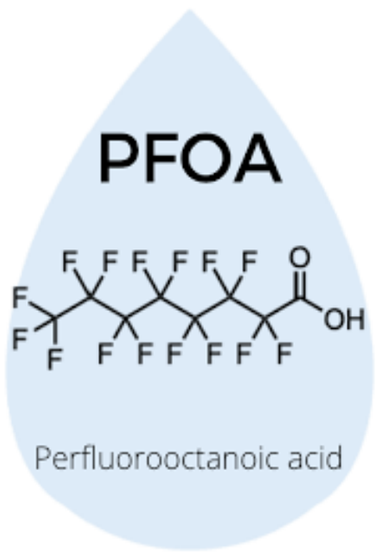
INTRODUCTION

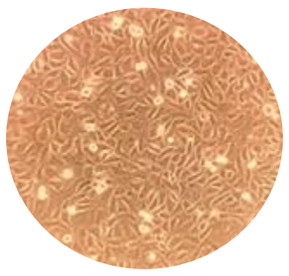
Perfluorooctanoic acid (PFOA), a synthetic chemical known for its persistence in the environment, is commonly used in various industrial and consumer products. PFOA is recognized for its toxic effects on the nervous, endocrine, and reproductive systems, while emerging research suggests it may also impact vascular function, potentially contributing to cardiovascular diseases. However, the mechanisms underlying PFOA-induced vascular dysfunction remain unclear. Notably, there is a lack of *in vitro* studies that accurately replicate real-life exposure scenarios.



"forever chemical"

The goal of this study was to investigate whether long-term low-level exposure to PFOA affects the survival of human endothelial cells *in vitro*.

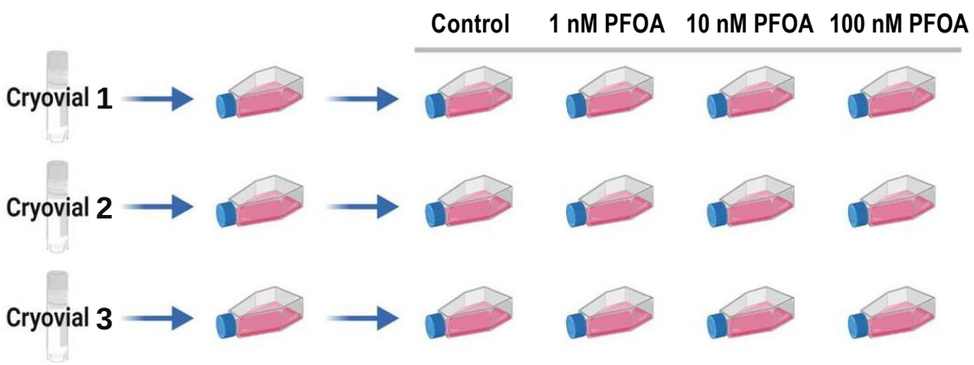
METHODS



EA.hy926
macrovascular endothelial cell line of human origin

EA.hy926 cells → monolayer cultures grown at 37 °C in a humidified atmosphere with 5% CO_2 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).

Cells originating from 3 different cryopreserved stock vials (biological replicates) were exposed to either control conditions (vehicle, 0.05% DMSO) or three human exposure relevant conc. of PFOA (1, 10, and 100 nM in 0.05% DMSO) and cultured independently in cell culture flasks for 12 weeks. The cells were passaged, counted, and exposed to either vehicle or PFOA twice a week.



Metabolic activity → alamarBlue™ assay

Cell viability → Trypan blue dye-exclusion test

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

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)

CONCLUSIONS

- No changes in metabolic activity and cell viability after 6 and 12 weeks of exposure to any of the three concentrations of PFOA
- A decrease in cell proliferation after 12 weeks of exposure to 100 nM PFOA
- Slightly decreased percentage of live cells and increased percentage of necrotic cells after 6 weeks of exposure to 1 nM PFOA
- Reduced percentage of live cells and increased percentage of necrotic cells after 12 weeks of exposure to all investigated concentrations of PFOA

In summary → prolonged exposure to low-level PFOA adversely affects EA.hy926 cell proliferation and negatively impacts cell survival by promoting apoptosis and necrosis.

RESULTS

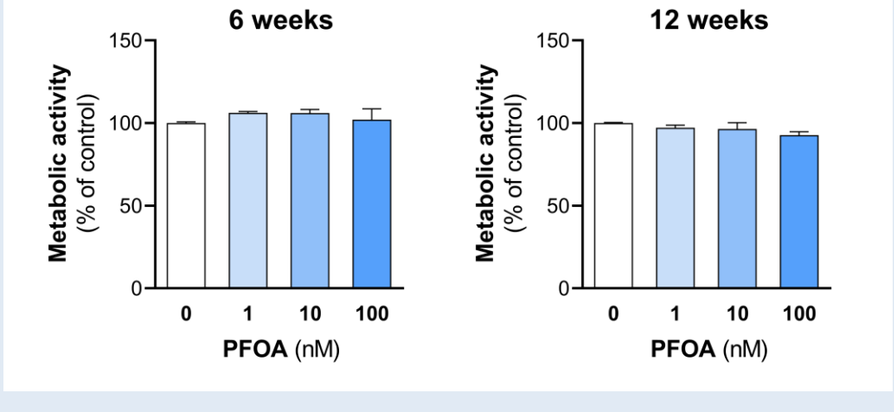


Figure 1. Metabolic activity was not affected after 6 weeks and 12 weeks of continuous exposure of EA.hy926 cells to 1 nM, 10 nM, and 100 nM PFOA.

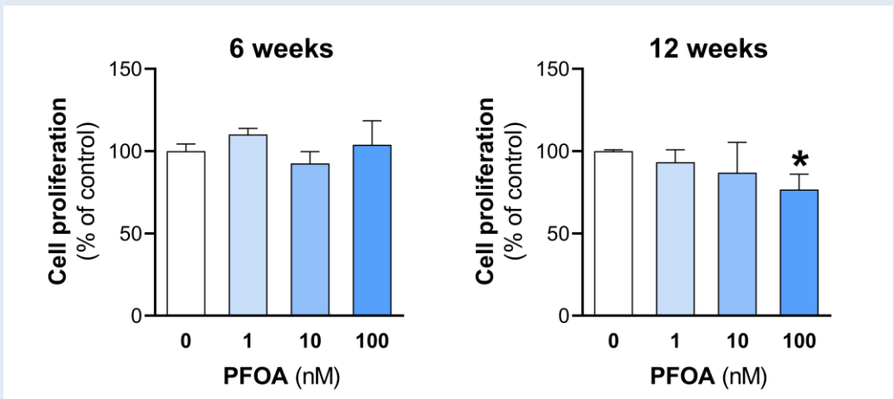


Figure 2. Cell proliferation was diminished after 12 weeks of continuous exposure of EA.hy926 cells to 100 nM PFOA.

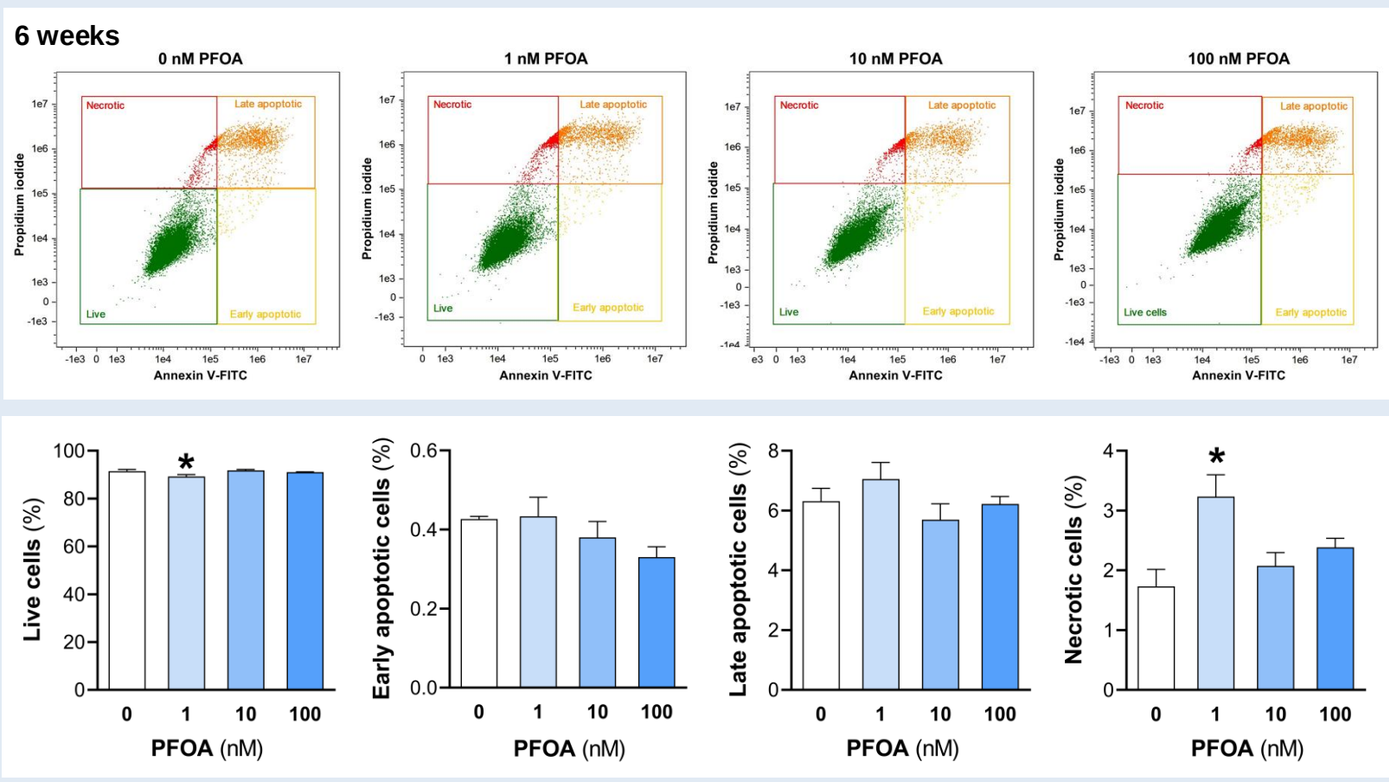


Figure 3. Increased percentage of necrotic cells and decreased percentage of live cells were observed after 6 weeks of continuous exposure of EA.hy926 cells to 1 nM PFOA, whereas the percentage of apoptotic cells was not changed upon exposure to any of the investigated concentrations of PFOA.

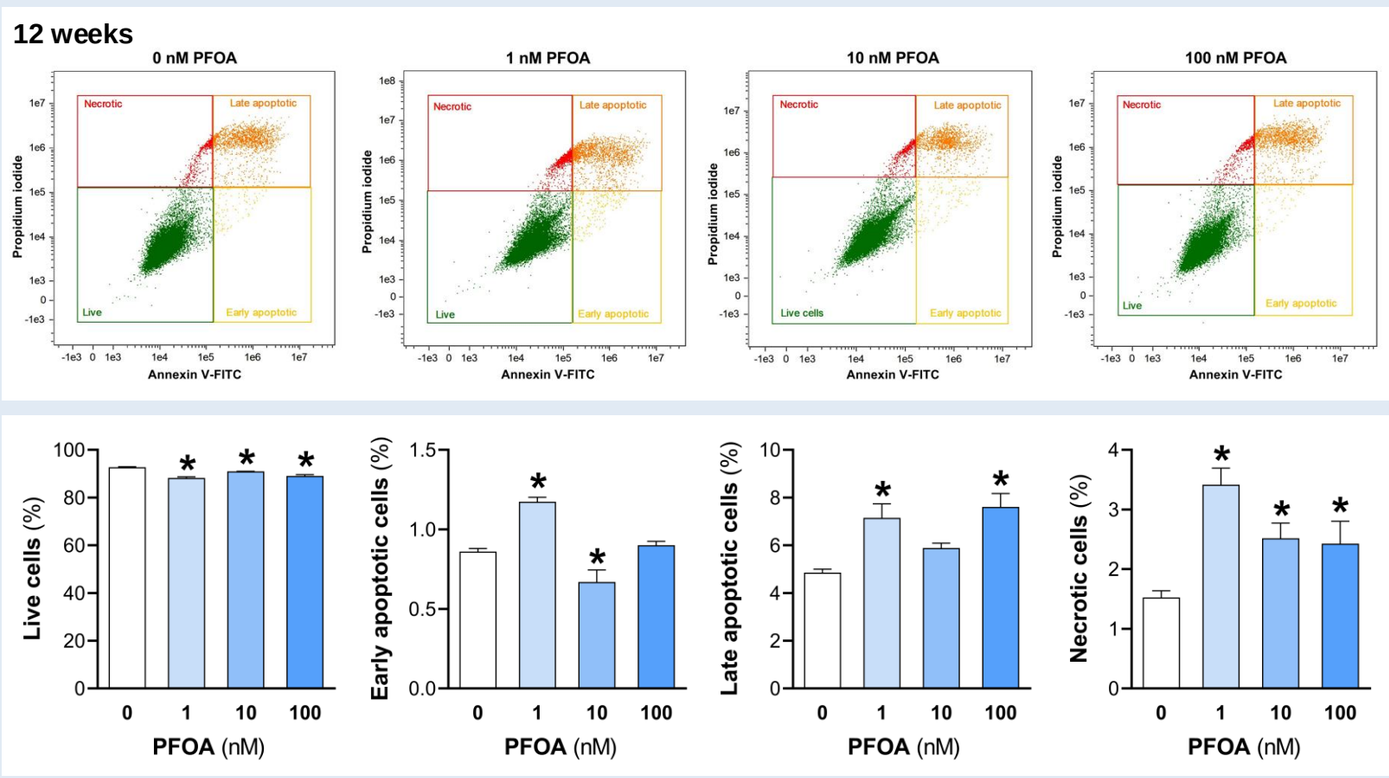


Figure 4. Decreased percentage of live cells and increased percentage of necrotic cells were observed after 12 weeks of continuous exposure of EA.hy926 cells to all three investigated concentrations of PFOA, whereas the percentage of apoptotic cells was most prominently increased upon exposure to 1 nM PFOA.

Acknowledgements

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