POLYSTYRENE NANOPLASTICS PRIME PLATELETS TO DRIVE PRO-INFLAMMATORY AND PRO-FIBROTIC ACTIVATION OF CARDIAC FIBROBLASTS

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Background and Aims. Nanoplastics (NPs) are emerging environmental contaminants with potential cardiovascular effects, but their impact on platelet activation and cardiac remodeling remains poorly defined. Atrial fibrosis is a key mechanism underlying atrial fibrillation (AF), driven by fibroblast transition to a pro-fibrotic phenotype. Platelets contribute to thrombo-inflammation and may infiltrate atrial tissue, modulating fibroblast phenotype. We investigated whether polystyrene NPs (PS-NPs, 50 nm) induce platelet activation in vitro and in vivo and enhance platelet-fibroblast crosstalk, promoting pro-inflammatory and pro-fibrotic signaling.

Methods. PS-NPs were administered intravenously in C57BL/6 mice, and serum thromboxane B_2 (TXB_2) was measured after 5 days as an index of platelet activation. Human isolated platelets were incubated with 50 nm PS-NPs (20 or 200 µg/mL) for 1 and 18 h at 37 °C under rotational conditions and assessed for TXB_2 generation and P-selectin membrane translocation. Washed human platelets were exposed to fluorescent Dragon Green-labelled particles, and platelet-derived extracellular vesicles (EVs) release and NPs incorporation into EVs were evaluated. Human atrial fibroblasts (HCF) were incubated for 24 h with platelet releasate obtained following platelet-NP interaction to assess effects on cytokine production and Snail expression.

Results. Single-dose i.v PS-NP administration (20 or 200 µg/mL) in C57BL/6 mice increased TXB_2 generation after 5 days (2.15- and 2.21-fold vs. baseline, $p<0.01$). Consistently, human platelets exposed to PS-NPs (20 or 200 µg/mL) for 1 and18h, showed a concentration-dependent significant increase of TXB_2 levels, even in the absence of platelet agonists. PS-NP exposure also significantly enhanced P-selectin surface expression at the highest concentration ($p < 0.05$). Exposure to PS-NPs did not significantly affect platelet-derived EVs release at 1 h or 18 h. However, the percentage of NP-positive EVs increased from ~45% to ~80% (20 vs. 200 µg/mL; $p < 0.01$), suggesting interaction and/or incorporation into EVs. To investigate functional consequences of platelet activation induced by PS-NPs, HCF cells were treated with platelet releasate collected after platelet incubation with PS-NP. After 24h, releasate from PS-NP unexposed platelets significantly upregulated the expression of inflammatory and remodeling markers, including interleukin (IL)-1α, IL-6, and IL-1β, as well as the fibroblast activation marker Snail. Notably, exposure of HCFs to releasate from PS-NP–treated platelets further enhanced the expression of inflammatory cytokines and Snail, with the strongest induction observed with releasate from platelets exposed to 200 µg/mL PS-NPs, indicating an additional effect of PS-NPs on platelet activation that translates into amplified fibroblast responses.

Conclusions. PS-NPs promote platelet activation and enhance platelet–fibroblast crosstalk, amplifying thrombo-inflammatory and pro-remodeling signaling. Although EVs release was unchanged, the increase in NP-positive EVs suggests their incorporation into EVs and a potential role in their systemic distribution. By priming platelets toward a more reactive phenotype, PS-NPs may contribute to atrial remodeling, supporting their role as an underrecognized modulator of cardiovascular pathophysiology.

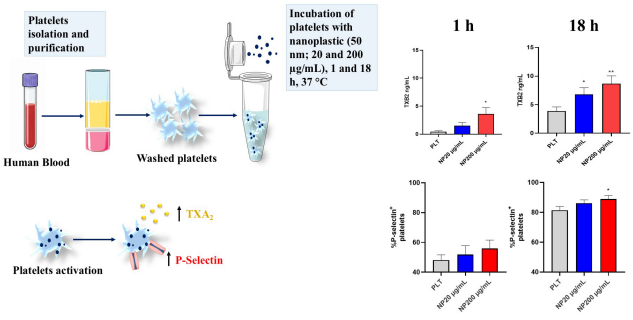


Figure 1 In the absence of platelet agonists, exposure to PS-NPs (50 nm; 20–200 µg/mL) induced a time and concentration-dependent increase in TXB_2 generation and P-selectin exposure in washed human platelets at 1 h and 18 h. * $P < 0.05$ and ** $P < 0.01$ for NP exposed platelets vs. control PLT.