# Nitric Oxide Prevents Human Platelet Adhesion to Fiber Membranes in Whole Blood

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**During cardiopulmonary bypass or long-term extracorporeal life support, foreign surface induced platelet deposition in the oxygenator causes deterioration of gas exchange. In this study, the authors evaluated the effectiveness of nitric oxide (NO) in reducing the adhesion of platelets in whole blood to the surface of hollow fiber membranes. For this purpose, a test chamber was designed consisting of a gas exchanger with ten Mitsubishi multi-layered composite hollow fibers (MHF: 257 mm OD; 203 mm ID; 70 mm length) and a polypropylene tube (16 mm OD; 100 mm length). Pure $N_2$ (control) or nitric oxide (NO) (100 ppm, 200 ppm in $N_2$) were delivered into the test chamber previously filled with 13 ml human whole blood. Platelet counts and platelet factor 4 (PF4), as a measure of platelet activation, were measured before and after either 1 or 2 hr of testing, and fibers were observed under scanning electron microscopic study (SEM) after each experiment. In the control and 100 ppm NO groups, platelet counts decreased and the level of PF4 increased during the 1 hr period. In the 200 ppm NO group, almost no platelet deposition could be observed on the surface of fibers under SEM. In conclusion, NO flow through hollow fiber membranes can markedly reduce platelet adhesion. Additional quantitative studies should define the optimal concentration for this effect and determine if this finding could improve oxygenator function, especially under conditions of long-term support. *ASAIO Journal* 1996;42: M850–M853.**

The need for a protective strategy for platelets during cardiopulmonary bypass and extracorporeal life support is based on the demonstration of platelet adherence and activation after blood contacts foreign surfaces.[1] Platelet activation leads to thrombin generation and clot formation that, even in the presence of heparin, results in microembolic complications and increased blood loss.

Nitric oxide (NO), first recognized as a humoral vascular relaxation factor,[2] is now known as a potent inhibitor of platelet aggregation and adhesion.[3,4] *In vivo*, NO diffuses directly from endothelial cells to inhibit platelets. This inhibition is associated with an elevation of intracellular guanosine 3',5'-cyclic monophosphate (cGMP) levels in the platelets.[5] In short, NO induced increases in cGMP initiate a number of cGMP dependent phosphorylation steps, finally leading to suppression of intracellular Ca2+ levels, whose sudden increase is a prerequisite for platelet activation.

We hypothesized that porous membrane fibers that continuously release exogenous NO would mimic vascular endothelium's nonadhesive and nonthrombogenic properties.

## Materials and Methods

### *Test Chamber*

The test chamber consisted of a gas exchanger and a polypropylene tube (16 mm OD; 100 mm length) whose surface is coated with human albumin solution (albumin 4.0 g/dl). The gas exchanger is made of ten Mitsubishi (Tokyo, Japan) multi-layered composite hollow fibers (MHF: 257 mm OD; 203 mm ID; 70 mm length) and a central gas conduit with two lumens. The inner lumen is connected to the gas source and conducts the gas to the tip of the device, where it is sucked through hollow fibers to the outer lumen, which is connected to a vacuum system.

### *Blood Preparation*

Blood (30 ml) drawn from normal, aspirin refraining human donors was placed directly into albumin coated polyethylene tubes with 0.6 ml of 0.5 M sodium citrate and directly transferred to the test chamber that was filled with approximately 13 ml citrated blood in a totally filled fluid system without any residual air.

### *Gas Administration Systems*

The systems configuration is shown as **Figure 1.** Nitrogen gas (Jackson Welding, Morgantown, WV) or nitric oxide gas, obtained in a mixture of nitrogen at 1,000 ppm NO (Scott Medical Gases, Lansing, MI), was delivered via a calibrated $N_2$ flow meter into the inner lumen gas transport tube of the gas exchanger. The concentration of nitric oxide gas was varied at 50, 100, and 200 ppm. Nitrogen gas and nitric oxide gas were delivered for 60 and 120 min, respectively. The concentration of nitric oxide gas was continuously measured by a Sievers (Boulder, CO) NOA 270 chemiluminescence system connected to the outer lumen gas transport tube, and sampled gas at a rate of 200 ml/min. During experiments, the test chamber was incubated and shaken at 37°C using a horizontal platform. The entire contents of the test chambers and fiber membranes were analyzed after the given times.

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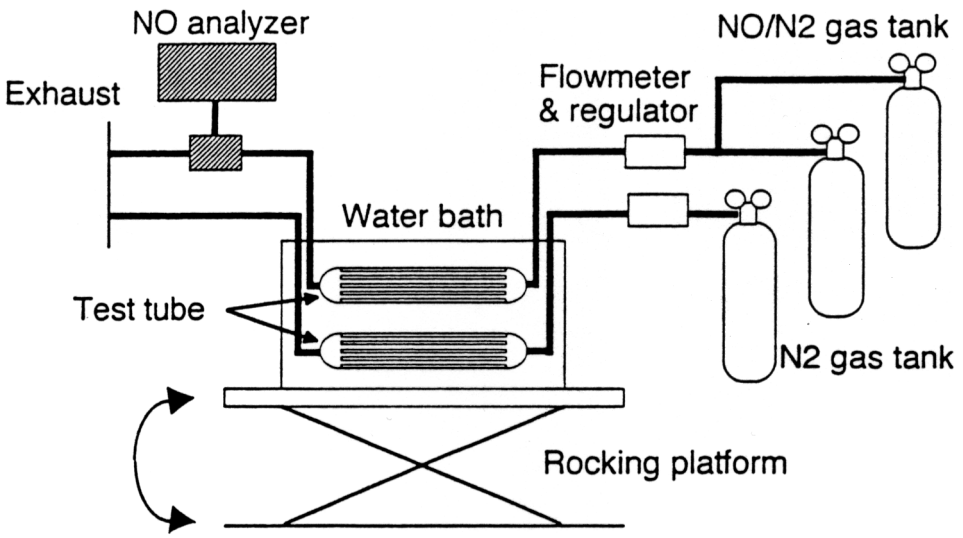


**Figure 1.** System configuration.

*Observable Parameters*

**Platelet Counts and Platelet Factor 4.** Platelet counts were measured before and after experiments using the automated laser cell counter. Platelet activation was evaluated by measuring changes in the plasma level of platelet factor 4 (PF4) using an enzyme immunoassay (ELISA) kit. Blood for PF4 (4.5 ml) was allowed to cool in the ice water bath and centrifuged at 2,500 *g* for 30 min. The resulting plasma supernatant was frozen (−70°C) until assayed.

**Nitrate $NO^{2-}$ + Nitrate $NO^{3-}$ Measurements.** Plasma levels for nitrate plus nitrite (the stable end products of NO) concentration were measured using an automated procedure based on the Griese reaction, as described previously.[6] The plasma levels for nitrate plus nitrite were measured before and after experiments, and the increased level of nitrate plus nitrite was calculated.

*Scanning Electron Microscopic Study*

Surfaces of the hollow fiber membranes were examined using scanning electron microscopic study (SEM). Fiber pieces were incubated for an hour in glutaraldehyde solution. After fixation, the segments were placed in phosphate buffered solutions (PBS), and serial dehydration steps were done with increasing concentrations of ethanol to remove all water from surfaces. Materials were critical point dried, and the specimens were coated with a microthin layer of metal and examined in a scanning electron microscope (JEOL T-300; Zeiss, Inc., New York, NY).

*Data Analysis*

Plasma levels are reported as means plus or minus the standard deviations. Comparisons between the different groups were performed by the Mann-Whitney test. Values of $p < 0.05$ or less were considered statistically significant.

## Results

The plasma level for nitrate plus nitrite accumulated in proportion to the concentration of delivered exogenous nitric oxide **(Figure 2).** This parallel increase in plasma levels of

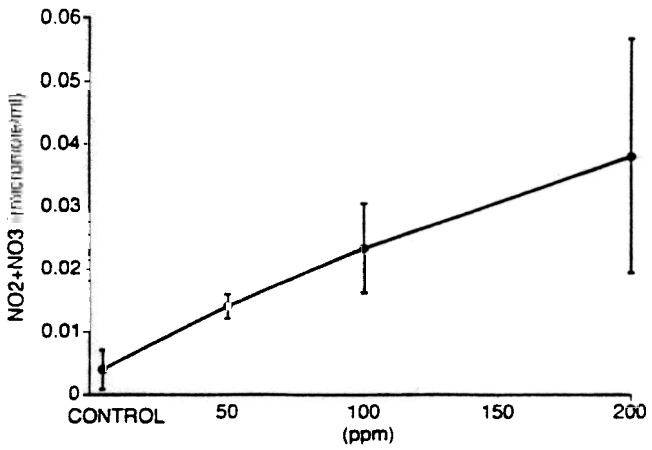


**Figure 2.** NO gas concentration *vs* accumulation of $NO^{2-}$ + $NO^{3-}$ in the blood.

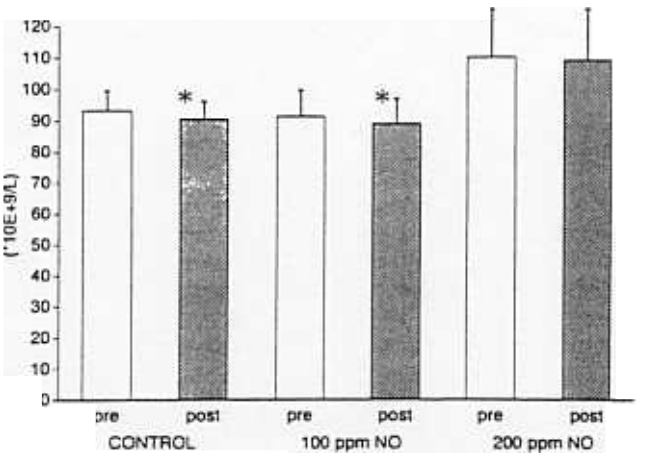


**Figure 3.** Platelet counts before and after incubation ($^*p < 0.05$).

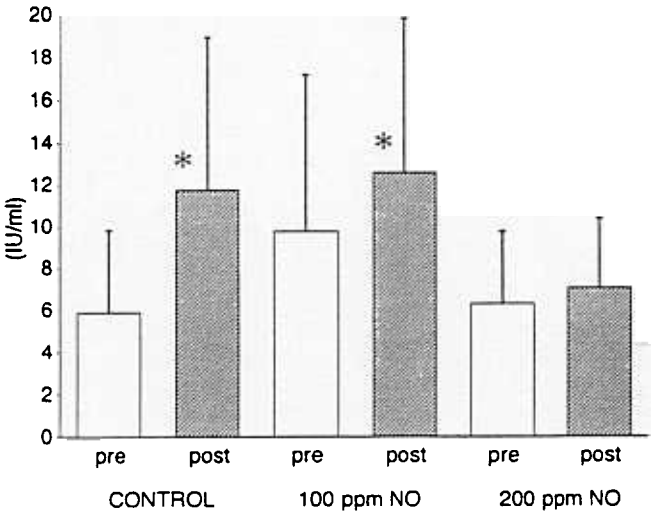


**Figure 4.** Level of PF4 before and after incubation (*$p < 0.05$).

nitrate plus nitrite clearly demonstrated NO transfer from the fiber membrane into the blood. Approximately 5% of delivered NO molecules were calculated to be oxidized to nitrate or nitrite in the test chamber in this study. The variation in platelet counts is shown in **Figure 3.** No significant differences were noted between before and after 200 ppm NO had been delivered for 60 min. However, a small but significant decrease in platelet count was shown for the 100 ppm NO group. During the 120 min period, platelet counts were decreased even in the presence of NO. Similarly, **Figure 4** shows that the level of PF4 showed no significant increase for the 200 ppm NO group during the 60 min period, whereas there was a significant increase in the level of PF4, regardless of the concentration of delivered nitric oxide gas, during the 120 min period. **Figure 5** shows that a significant ($p < 0.001$) negative correlation existed between the increased plasma level of nitrate plus nitrite and the level of PF4 during the 60 min experiment.

SEM revealed that substantial platelet deposition could be observed on the hollow fiber membranes in the absence of NO **(Figure 6B),** in contrast with the inhibitory effect of 200 ppm NO on platelet deposition on the surface of fibers **(Figure 6A).** This observation was consistent in all fibers using higher concentrations of NO, whereas at 100 ppm platelet deposition on the membranes could be seen.

## Discussion

The results of this study demonstrate that exogenous nitric oxide gas significantly inhibits platelet adhesion to the surface of hollow fiber membranes exposed to whole blood. Nitric oxide, which was first discovered as a endothelium derived relaxing factor (EDRF),[2] now is recognized as a potent inhibitor of platelet aggregation and adhesion. Because NO is rapidly inactivated by hemoglobin,[7] the contributions of NO to the nonadhesive properties of vascular endothelium were demonstrated in a number of reports using endothelium exposed to washed platelets.[3,4] Likewise, in our study, NO molecules were inactivated by hemoglobin as soon as they were transferred into blood. NO molecules have an ultrashort half-life of 3–5 sec because of their oxidation to nitrate and nitrite. Nevertheless, NO molecules could act as an inhibitor of platelet activation or adhesion at a boundary between the surface of fiber membranes and whole blood, which would limit their reactive properties to the membrane.

The increased plasma level of nitrate and nitrite was directly correlated to the concentration of delivered exogenous nitric oxide gas **(Figure 2)** and the duration of testing time. Although we did not measure the plasma level of methemoglobin (MetHb) and nitrosyl hemoglobin (HbNO) in the current study, MetHb and HbNO and nitrate and nitrite could accumulate, as Wennmalm *et al.*[8] have reported that NO is almost quantitatively converted to MetHb in arterial blood, whereas more HbNO and less nitrate are formed in venous blood.

The change in platelet count was chosen for evaluation of the amount of platelet deposition. Because the calculated whole surface area of these fiber membranes of the gas exchanger was too small compared to the total amount of platelets in whole blood, the change in platelet count might not be a sensitive method for evaluation of the amount of platelet deposition.

The plasma level of PF4, a marker of platelet activation, did not increase in the 200 ppm NO group during the 60 min of testing, although it significantly increased in all groups during 120 min of testing. This corresponded with the semiquantitative observation under SEM in which a lack of platelet deposition at higher NO concentrations was consistently seen. The negative correlation between the plasma level of nitrate plus nitrite and the level of PF4 suggests that higher doses of NO may prevent platelet activation.

## Conclusions

Exogenous NO as part of the gas mixture may diminish platelet activation and adhesion to the surface of fiber membranes. In the future, a quantitative study should be designed and the optimal concentration of NO for this protective effect identified.

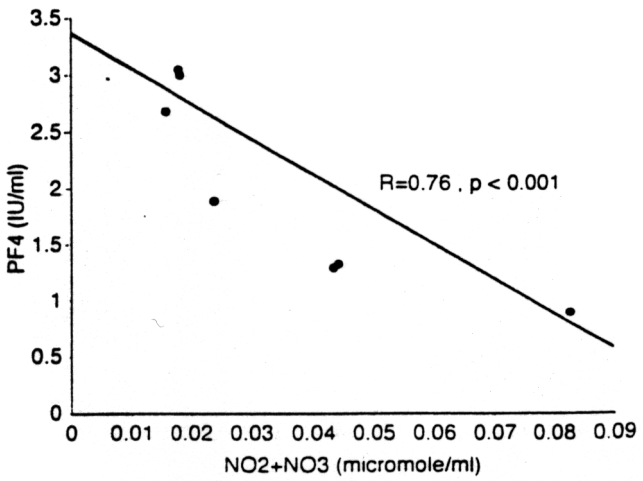


**Figure 5.** Correlation between $NO^2$ + $NO^3$ .

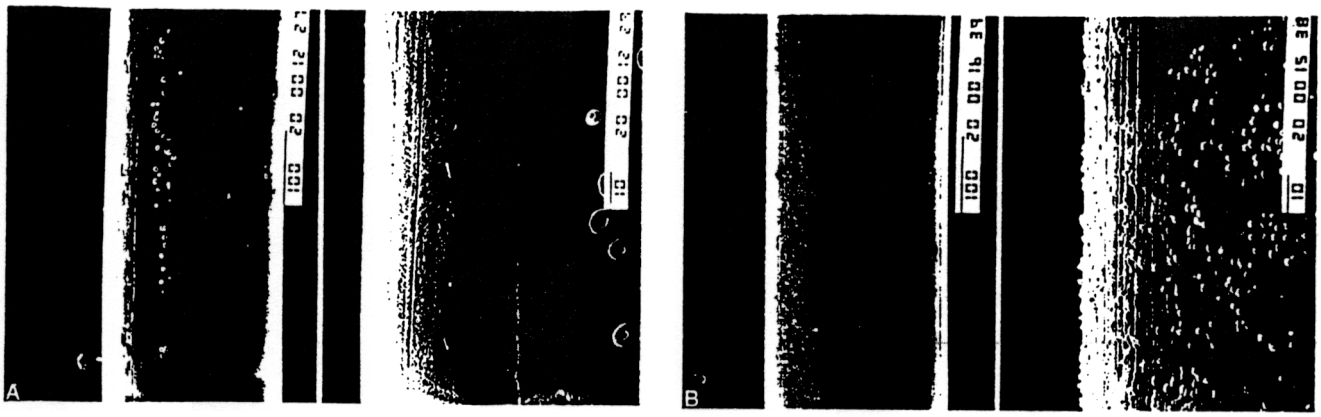

**Figure 6.** Surface of fiber membrane by SEM. (A) NO 200 ppm. (B) $N_2$.

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