Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Fibrinogen-erythrocyte binding and hemorheology measurements on the assessment of essential arterial hypertension patients

Ana Filipa Guedes[1], Carlos Moreira[2], José Braz Nogueira[2], Nuno C. Santos[1,*], Filomena A. Carvalho[1,*]

Linear regression analysis between whole blood viscosity and AFM data

Trying to understand how the interactions between fibrinogen molecules and its erythrocyte membrane receptor could affect the whole blood viscosity of EAH patients' group, data obtained for each parameter were compared (Figure S1). After performing linear regression analysis, we could observe a significant relation between blood viscosity after the correction of hematocrit, at 225 s^{-1} shear rate, and the average fibrinogen-erythrocyte (un)binding force (p = 0.017; r^2 = 0.20). We also obtained significant relations for native blood viscosity at both shear rates (p = 0.0002 for 22.5 s^{-1}, r^2 = 0.40; p = 0.005 for 225 s^{-1}, r^2 = 0.25). This means that increasing the force of fibrinogen-erythrocyte binding, blood viscosity also increases.

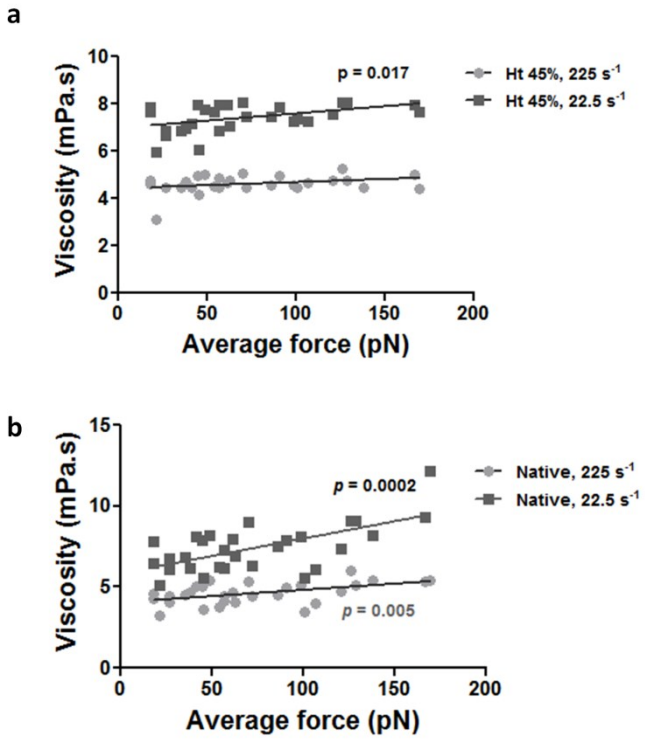

Figure S1 | **Relation between fibrinogen-erythrocyte binding force and whole blood viscosity for EAH patients.** Linear regression analysis between fibrinogen-erythrocyte average (un)binding force data and whole blood viscosity, for 45% hematocrit data **(a)** and native blood data **(b)**. Linear regression was performed with a confidence interval of 95%. We observed a significant relation between native whole blood viscosity at both shear rates and binding force, as well as between 45% hematocrit blood viscosity at 22.5 $s^{-1}$and binding force. Statistical significance values are shown.

Linear regression analysis between fibrinogen concentration and AFM data

In an effort to understand why the AFM data show that CHF patients have significantly increased fibrinogen-erythrocyte binding force, we evaluated the relationship between the fibrinogen levels in plasma from each EAH patient and binding force (Figure S2). We observed that increasing fibrinogen concentration, the average binding force (measured at the single-molecule level) also increases (p = 0.047, r^2 = 0.14). High fibrinogen plasma levels on EAH patients could lead to high binding strength between this protein and its erythrocyte membrane receptor. Thus, these findings support the idea

that fibrinogen has an important role on promoting erythrocyte aggregation, increasing the propensity for cardiovascular events.

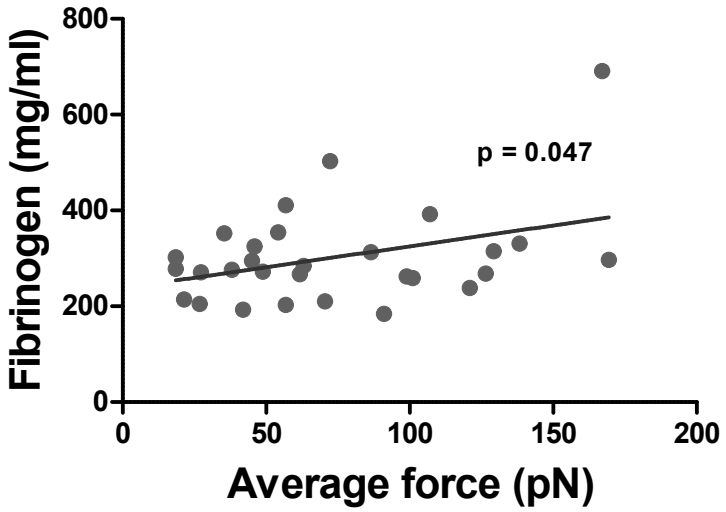

Figure S2 | Relation between fibrinogen-erythrocyte binding force and plasma fibrinogen concentration for EAH patients. Linear regression analysis showed a significant relation between fibrinogen-erythrocyte average (un)binding force data and plasma fibrinogen levels data. Linear regression was performed with a confidence interval of 95%. Statistical significance was $p = 0.047$.

Linear regression analysis between LDH and AFM data

Lactate dehydrogenase (LDH) blood levels are clinically used on the evaluation of a number of diseases and conditions. It is a general indicator of and cellular lysis, releasing the enzyme to the extracellular medium. Considering the tissue-specifity of the different isoforms of this enzyme, LDH is also used to help diagnose and monitor a heart attack. In our study, high LDH levels were observed for EAH group. To understand if there is any association between this parameter and the binding force between fibrinogen and erythrocytes, we performed a linear regression analysis (Figure S3). We observed a positive relation between fibrinogen-erythrocyte binding force and LDH data, showing

that in EAH patients, increasing the LDH circulating levels, also increases the binding force (p = 0.023, r^2 = 0.21).

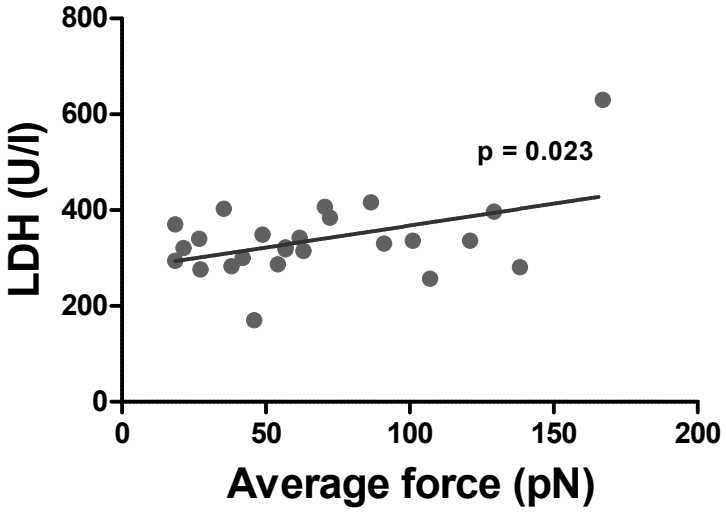

Figure S3 | Linear regression shows the relation between fibrinogen-erythrocyte binding force and plasma LDH levels. A significant relation was found between fibrinogen-erythrocyte average (un)binding force data and plasma LDH data. Linear regression was performed with a confidence interval of 95%. Statistical significance was p = 0.023.