AFM as a nanotool to evaluate protein-cell interactions and cell-cell adhesion on cardiovascular pathologies

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Abstract

Increased levels of plasma fibrinogen result in changes in blood rheological properties, which are not completely clarified [1,2]. Erythrocyte aggregation has become an issue of increasing interest, especially as an indicator of the associated cardiovascular risk, since it is influenced mostly by fibrinogen levels [2-4]. A better understanding of the role of fibrinogen on erythrocyte aggregation in cardiovascular pathologies patients may be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions. Our previous studies [5,6] demonstrated the existence of a single-molecule interaction between fibrinogen and a receptor on the erythrocyte membrane, with a lower but comparable affinity relative to platelet binding. The receptor identified is not as strongly influenced by calcium and eptifibatide (an $\alpha_{IIb}\beta_3$ specific inhibitor) as the platelet receptor. The results from Glanzmann thrombastenia (a rare hereditary bleeding disease caused by $\alpha_{IIb}\beta_3$ deficiency) patients showed, for the first time, an impaired fibrinogen-erythrocyte binding. Correlation with genetic sequencing data demonstrated that one of the units of the fibrinogen receptor on erythrocytes is a product of the expression of the β_3 gene. More recently, we also demonstrate that younger erythrocytes may be the main cells responsible for some cardiovascular diseases associated with an increase on the fibrinogen content in blood [7].

The aim of this study was to understand how fibrinogen influences erythrocyte aggregation by cell-cell adhesion force spectroscopy measurements using an atomic force microscope (AFM). Additionally, we evaluated how this protein-cell interaction constitutes a cardiovascular risk factor in different cardiovascular pathologies.

Cardiovascular patients with heart failure (HF; N=30), essential arterial hypertension (EAH; N=31) and aortic stenosis (N=25), as well as 15 healthy blood donors were engaged in this study. HF patients were grouped according to two etiologies: ischemic or non-ischemic HF. Fibrinogen-erythrocyte binding measurements were conducted by AFM-based force spectroscopy, in buffer, with the protein covalently attached to the AFM tip. Erythrocyte-erythrocyte measurements were conducted only for healthy subjects, with one of the cells attached to AFM tipless cantilevers and the other on the solid substrate. Erythrocyte-erythrocyte adhesion forces were measured in the absence and in the presence of increasing fibrinogen concentrations.

Cell-cell adhesion data showed that increasing fibrinogen concentrations there is an increase in the work necessary for cell detachment, from 0.45 ± 0.04 fJ without fibrinogen to 12.0 ± 0.13 fJ at 1 mg/ml fibrinogen (p<0.001) (Figure 1A). Concomitantly, average cell-cell detachment forces increase from 72.0 ± 2.9 pN without fibrinogen to 250.4 ± 3.2 pN at 1 mg/ml fibrinogen (p<0.001) (Figure 1B). We also observed a 3.5-fold increase on the number of membrane tethers per curve on the cell-cell detachment in the presence of fibrinogen 1 mg/ml, comparing with the experiments without fibrinogen. AFM data allow, for the first time, the quantification of the adhesion force necessary to detach two erythrocytes in the presence of different concentrations of fibrinogen. Our *in vitro* study tried to mimic what happens *in vivo* on the human blood flow. The results confirm that increasing fibrinogen plasma levels are associated with the higher tendency of erythrocytes to aggregate, probably by transient simultaneous binding of the protein to two cells, bridging them. This transient aggregation impairs blood flow and is associated with a higher risk of cardiovascular diseases.

Regarding the protein-cell interaction results, all cardiovascular patients presented significantly higher binding forces than healthy donors, despite lower binding frequency (Figure 2). HF ischemic patients presented higher forces than the non-ischemic ones (74.9 ± 10.7 pN *vs.* 45.4 ± 5.6 pN; p=0.021). Fibrinogen-erythrocyte interactions were higher in all cardiovascular patients than the control group. This could lead to changes on whole blood flow, representing a cardiovascular risk factor. EAH patients seem to have a higher cardiovascular risk dependent of the increase of fibrinogen plasma concentration levels. The results are relevant to conclude on the degree of pathophysiological relevance of fibrinogen and erythrocyte aggregation, since an increment on both might induce a state of microcirculatory slower flow, increasing the probability of cardiovascular complications.

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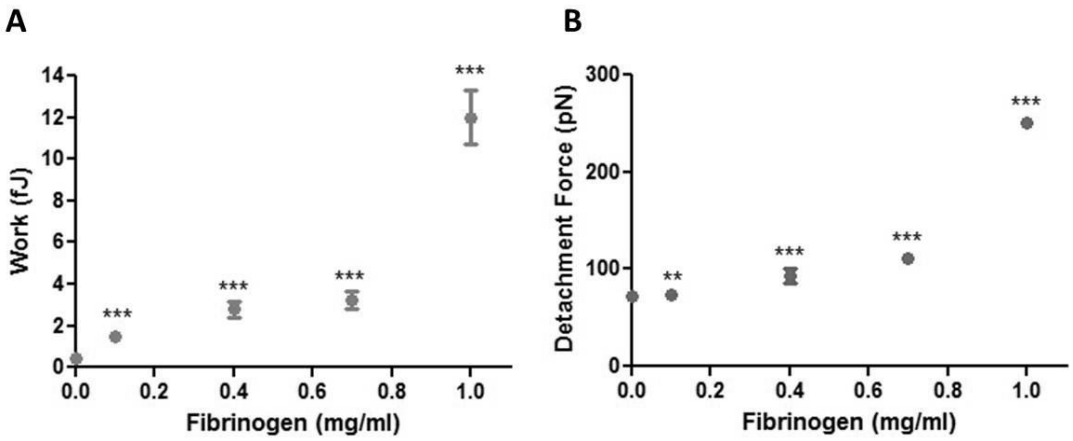

Figure 1: Cell-cell adhesion studies. Erythrocyte-erythrocyte adhesion in the absence and in the presence of increasing fibrinogen concentrations, measured by AFM-based force spectroscopy. Quantification of the work (A) and the detachment force (B) necessary to break the interaction.

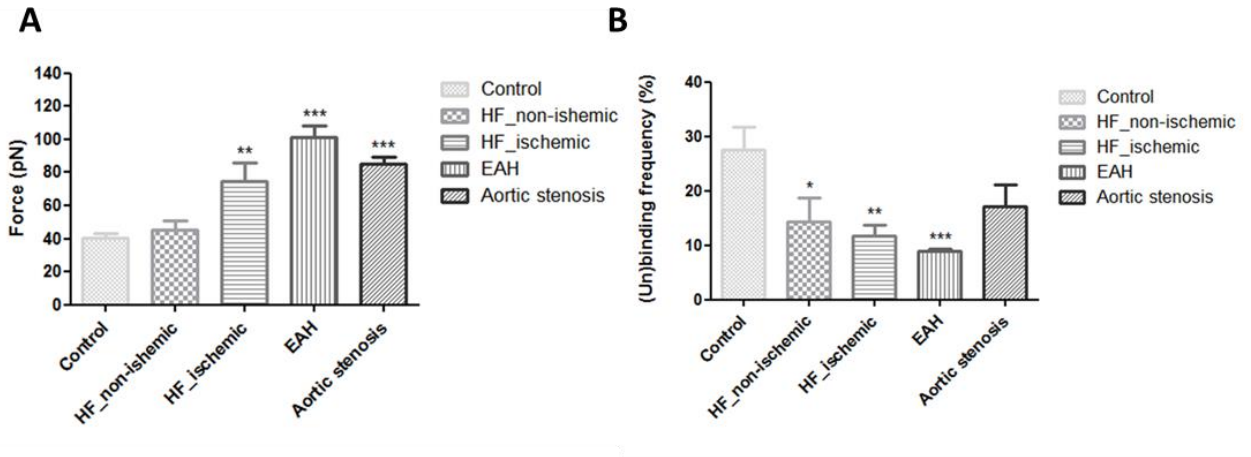

Figure 2: AFM-based force spectroscopy data of the interactions between fibrinogen and erythrocytes from patients with different cardiovascular pathologies, and healthy blood donors (control subjects). Average values of force (A), and percentage of (un)binding events (B) for all groups of patients and control. Data indicates that all groups of patients – heart failure (HF), essential arterial hypertension (EAH) and aortic stenosis – have an increase on the force of the binding between fibrinogen and erythrocyte, despite their decrease on (un)binding frequency (probability).