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Comparison of ultrastructural and nanomechanical signature of platelets from acute myocardial infarction and platelet activation

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ABSTRACT

Acute myocardial infarction (AMI) initiation and progression follow complex molecular and structural changes in the nanoarchitecture of platelets. However, it remains poorly understood how the transformation from health to AMI alters the ultrastructural and biomechanical properties of platelets within the platelet activation microenvironment. Here, we show using an atomic force microscope (AFM) that platelet samples, including living human platelets from the healthy and AMI patient, activated platelets from collagen-stimulated model, show distinct ultrastructural imaging and stiffness profiles. Correlative morphology obtained on AMI platelets and collagen-activated platelets display distinct pseudopodia structure and nanoclusters on membrane. In contrast to normal platelets, AMI platelets have a stiffer distribution resulting from complicated pathogenesis, with a prominent high-stiffness peak representative of platelet activation using AFM-based force spectroscopy. Similar findings are seen in specific stages of platelet activation in collagen-stimulated model. Further evidence obtained from different force measurement region with activated platelets shows that platelet migration is correlated to the more elasticity of pseudopodia while high stiffness at the center region. Overall, ultrastructural and nanomechanical profiling by AFM provides quantitative indicators in the clinical diagnostics of AMI with mechanobiological significance.

1. Introduction

Acute myocardial infarction (AMI), commonly known as a heart attack, can cause tissue damage and can even be life-threatening [1]. Studies show thrombosis is the important inducing factor in the development of AMI, which is related to the abnormal phenomenon of blood components, blood vessels and blood flow [1,2]. Platelets are circulating cellular sensors that play a critical role in thrombosis [3,4]. Once the vessel is injured, exposed collagens can stimulate platelet activation, leading to the alterations in their shape, adhesion, aggregation, and subsequently thrombus formation [5].

At the molecular level, AMI initiation and progression are accompanied by the complex structural changes in the extracellular matrix and collagen stimulus [6]. Collagen is known as the most agonist for stimulating platelet activation [7]. Once the extracellular matrix on the vessel is damaged, platelets rapidly bind to the collagen, resulting in platelet activation [8]. GPIIIa (called CD61), a kind of collagen receptor expressing on platelet membrane, plays a vital role in regulation of platelet activation in the human body [9]. Collagen specifically binds to CD61 with high affinity and stimulates resting platelets activation, which is closely related to AMI initiation [10].

The nanostructural changes of activated platelets are sensitively responsible for the stimuli from pathological environment [11–13].

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The changes in ultrastructure, elasticity and/or deformability are accompanied by alterations in the cytoarchitecture that have known associations with platelet activation, migration and aggregation [13]. Studies demonstrate the morphological transitions of platelets are strongly depended on their elastic properties of membrane and cytoskeleton [14]. However, the correlating biomechanical and ultrastructural properties for platelets at different activated stages are still poorly understood, because nanoscalar information at physiological condition isn't obtained by traditional technologies. Conventional techniques, including fluorescence microscopy [14] and scanning ion conductance microscopy [15,16], have been used to investigate platelets, whereas they have limitations of time-consuming sample preparation, fluorescent labeling, un-physiological imaging condition and low resolution.

Atomic force microscopy (AFM), a force-dependent technique, is used to obtain ultrastructural and nanomechanical properties of cells or clinical samples [17–20]. AFM-based force spectroscopy found that cancer cells isolated from the patients' tissue are softer than normal cells [21–24], showing that nanomechanical profiling by AFM can provide quantitative indicators in the clinical diagnostics. Platelets are suited to be investigated by AFM-based nanomechancial characterization because of their soft membrane structure [15]. Leong et al. showed platelets extracted from AMI patients had high thrombin level and increased Young's modulus in comparison with the healthy, confirming the strong relationship between AMI and thrombin [25]. Du Plooy et al. investigated the platelet stiffness in blood samples taken from thrombo-embolic ischemic stroke, the membrane stiffness from the patients' group was larger than that of the healthy group [15]. Liang et al. found that platelets could govern the retraction and stiffen the blood clots through generation of active contractile forces, which was essential for blocking thrombosis [26]. Although those studies have shown the nanomechanical changes in pathological platelets, the ultrastructural imaging and nanomechanical profiles for AMI platelets, resting platelets and activated platelets have rarely reported to the best of our knowledge, in particular the correlation of collagen-stimulated activation model with AMI.

In this article, we report on a comprehensive effort to correlate the ultrastructural and nanomechanical properties of native platelet samples in the healthy, AMI patients and collagen-activated model by AFM. These findings show unique ultrastructural properties and mechano-markers that can be used to discriminate normal and AMI platelets, and suggest close correlations between collagen stimulus, platelet activation, AMI initiation and progression.

2. Experimental section

See Supplementary Data.

3. Results and discussion

3.1. Comparative study on ultrastructural and nanomechanical signature of normal and AMI platelets

Morphological and ultrastructural characteristics of platelets are closely related to their physiological status [27]. As shown in invert optical image, platelets from the healthy donor had a good dispersion (Fig. 1a), while platelets from AMI patient were aggregated around as the granular deposit on substrate (Fig. 1b). Typically, normal platelet had the circular structure with the diameter of 2–3 μm and the height of 350–800 nm (Supp. Fig. 1a), in accordance with the reported literature [16]. For AMI sample, AFM imaging illustrated a number of platelets are aggregated together (Fig. 1b), importantly, extended pseudopods, irregular aggregations and nanocluster structure on membrane surface were observed, suggesting that platelets were activated in AMI sample. Compared with normal platelet, a larger diameter and a larger height were measured from AMI platelet (Supp. Fig. 1b), implying the alteration in its shape was caused by the change in biofunctionalities.

Fig. 1c shows the difference in membrane roughness (including R_a and R_q) of AMI platelets compared with the normal. For the normal platelet, $R_a = 22.41 \pm 1.07$ nm and $R_q = 18.32 \pm 1.39$ nm were measured, whereas $R_a = 25.46 \pm 1.93$ nm and $R_q = 20.28 \pm 2.26$ nm were observed in AMI platelet, demonstrating AMI platelet had increased roughness in comparison with normal platelets. In the view of morphological and ultrastructural changes on platelet membrane, platelets from AMI patients show the significant difference with normal platelets, which is important to discriminate different clinical platelet samples at nanoscalar level.

Membrane elasticity is an important factor relating to cell function, adherence, migration, transformation and invasion [12]. To elucidate and correlate the respective nanomechanical profiles to pathogenetic findings in normal and AMI platelet, we carried out AFM-force spectroscopy analysis under physiological buffer conditions. The experimental approach for obtaining Young's modulus stiffness maps across platelet sample is described in Fig. 1d and e. Plotting a histogram of stiffness values from a normal platelet revealed a stiffness distribution of 1.82 ± 0.03 MPa. In comparison, a representative AMI platelet typically exhibited a stiffness distribution with peak at 2.18 ± 0.03 MPa. At values stiffer than 0.3 MPa, it indicates a marked mechanical enhancement for platelet from AMI patient. The correlation of nanomechanical profiles with matching histology indicates that the soft property is typical for normal platelet, whereas the stiffer value is for AMI platelet. The MPa of magnitude for this nanomechanical measurement is higher than that of kPa, which is ascribed to fixation procedures for sample preparation [15]. In this study, platelet samples are fixed before nanomechanical analysis because platelets are easily activated by the stimulus in external environment [4]. Therefore, the comparison in elastic properties between normal and AMI groups is not affected because the effects of fixed steps are the same in those two groups. Stiffer profiles presumably represent platelets are activated and correspondingly membrane elasticity is altered in AMI.

3.2. Relevance of collagen concentrations in ultrastructural response for platelet activation model

Collagen, the main component of extracellular matrix, can stimulate platelet activation [28]. Platelets in their activated conditions are known to promote thrombosis progression and subsequently induce AMI initiation [1,4]. Therefore, considering the diversity in patient samples, we turned to collagen-stimulated platelet activation, a reliable activation model, to more systematically elucidate morphological and ultrastructural properties of different activated stages for platelets. Furthermore, we compared the relation of ultrastructural and nanomechanical properties between collagen-stimulated platelet activation model and AMI pathogenesis. Results from fluorescent confocal imaging (Supp. Fig. 2) and flow cytometry (Supp. Fig. 3) demonstrated that CD61 expression was significantly enhanced after increasing collagen concentrations, and platelet activation progression was strongly relative to collagen stimulus.

AFM is used to comparatively investigate morphological and ultrastructural differences in resting platelets and collagen-activated model (Fig. 2). In the control group, the diameter of resting platelet was 2–3 μm, and the height was 0.7–1.0 μm (Fig. 2a). No filopodia structure was observed on resting platelet surface, indicating platelet was not activated. After the treatment of

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