

Morphological and nanomechanical properties of platelets derived from women with early pregnancy loss

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BACKGROUND

Platelets play a fundamental role in hemostasis. The process of hemostasis is a complex protective mechanism, related to the balance between the pro- and anticoagulation system and the fibrinolytic system. During normal pregnancy, this balance is strongly altered toward hypercoagulation state. A progressive increase in the concentration of pro-coagulant proteins is observed in the blood plasma in all stages of pregnancy. At the same time the natural anticoagulants and fibrinolytic agents are greatly reduced. In parallel markers of platelet's activation are elevated throughout pregnancy. The altered haemostatic status suggests an increased risk of thrombosis, especially in the uteroplacental circulation. This risk is elevated many times in women with thrombophilic mutation.

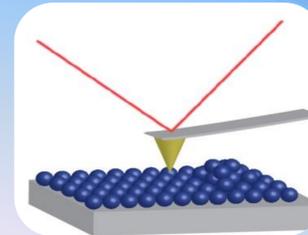
Recently, atomic force microscopy (AFM) was applied to investigate the biomechanical characteristics and 3-dimensional changes in platelet architecture on activation. The experiments on elasticity and morphology provide a new insight into the cell biomechanics.

AIM

To assess the changes in the morphological and nanomechanical properties (Young's modulus) of platelets derived from women with early pregnancy loss (EPL) and control pregnant (CP) and non-pregnant (CNP) women and to evaluate the impact of the carriage of polymorphisms in thrombophilia genes.

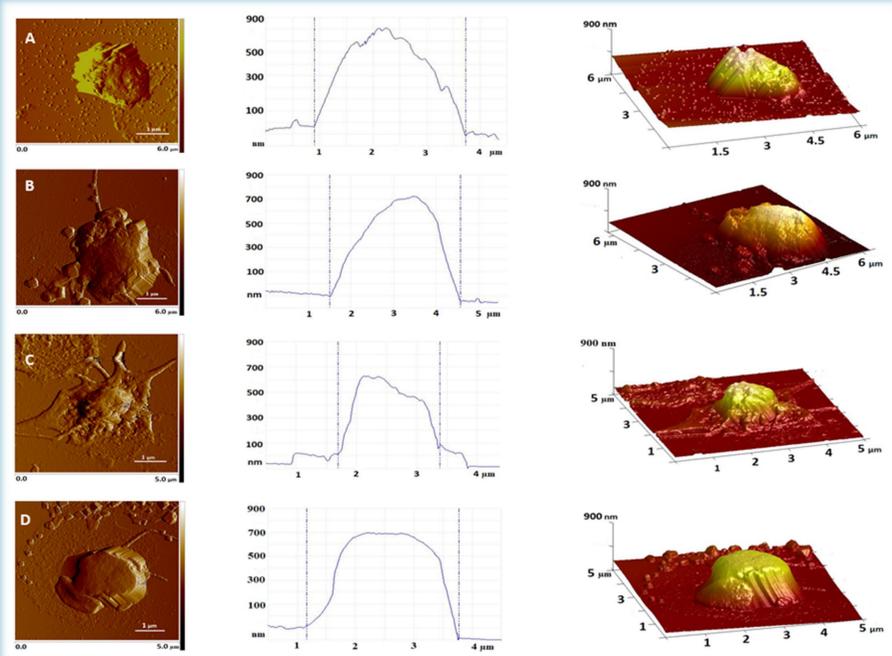
METHOD

Atomic force microscopy (AFM)



AFM provides quantitative imaging of different types of cells on nanometer scale and detects the roughness (distribution of heights of all points) and the mechanical properties of the imaged objects. AFM was performed with NanoScopeV system (Bruker Inc.)

RESULTS

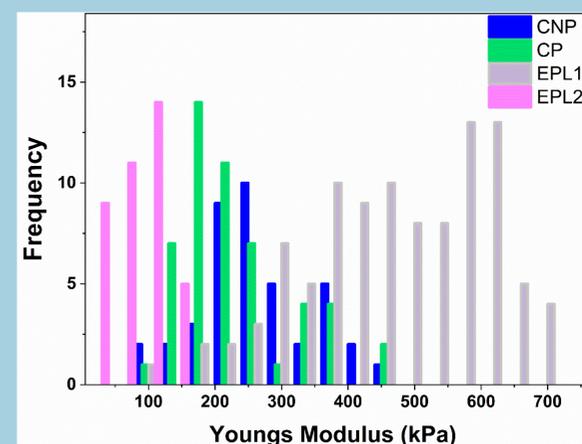


Representative AFM images of platelets derived from control non-pregnant (A) and pregnant (B) women and women with early pregnancy loss EPL1 (C -- embryonic stage of gestational development) and EPL2 (D - fetal stage of gestational development) deposited onto glass coverslip. The images were taken in tapping mode in air, at room temperature. The first column presents 2D height profiles with a shadowing effect, the second one - cross-section plot profiles and the third - 3D topographical images of the images in A, B, C and D, respectively.

Average values and standard deviations of the Height (H), Spreading Area, Roughness (Rrms), and Young's Modulus (Ea) of platelets derived from control non-pregnant (CNP) and pregnant (CP) women and women with early pregnancy loss (EPL1/ EPL2)

| Groups | H (nm) | Area (μm ²) | Rrms (nm) | Ea (kPa) |
|---|------------|-------------------------|-----------|-----------|
| CNP | 1090 ± 131 | 4.3 ± 1.3 | 39.1 ± 8 | 241 ± 103 |
| CP | 955 ± 88 | 4.8 ± 1.2 | 28.9 ± 6 | 174 ± 77 |
| EPL1 (with polymorphism in FVL, FII20210A, PLA1/A2, MTHFR or PAI-1) - embryonic stage | 683 ± 131* | 3.8 ± 1.0* | 24.8 ± 8 | 482 ± 98* |
| EPL2 (with polymorphism in PLA1/A2 or FVL) - foetal stage | 793 ± 131 | 5.4 ± 1.1* | 22.9 ± 6* | 68 ± 42* |

* Statistical significances between means were assayed using Student t test (P < 0.05).



Young's modulus histogram of platelets derived from control non-pregnant (A) and pregnant (B) women and women with early pregnancy loss EPL1 (C -- embryonic stage of gestational development) and EPL2 (D - fetal stage of gestational development)

Main morphometric and mechanical characteristic of platelets derived from women of EPL1 and EPL2 groups

| EPL1 groups | EPL2 groups |
|--|--|
| <ul style="list-style-type: none"> ➤ Reduced height and area of spreading; ➤ Elevated membrane stiffness compared to the control groups. | <ul style="list-style-type: none"> ➤ Increased area of spreading ; ➤ Strongly reduced membrane roughness and stiffness compared to the control groups. |

Conclusions:

- ❖ The platelets derived from women with EPL, in which the abortion occurred at embryonic stage of gestational development, are at later stage of activation compared to those from control pregnant and non-pregnant women.
- ❖ The AFM data demonstrate that the structural and mechanical variations in the platelets from women with EPL correlate with platelet hyperactivity that is associated with the carriage of polymorphisms in thrombophilia genes and the stage of gestational development at which the abortion occurred.
- ❖ This study highlights the potential of AFM technique to detect new pathologies.

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