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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Red Blood Cell Aggregation within a Blood Clot Causes Clot Shrinkage

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Volumetric shrinkage of blood clots known as clot contraction (retraction) driven by the activated platelets is important for hemostasis and thrombosis. RBCs make up more than a half of the volume of a blood clot, but their possible contribution to clot contraction is unknown, especially in severe thrombocytopenia and/or thrombocytopathies. Remarkably, in whole blood, unlike in platelet-rich plasma, platelet inhibitors never suppress clot contraction completely, suggesting a residual active mechanical contribution from RBCs. This study aims at elucidating the ability of RBCs *per se* to promote clot shrinkage and explore the underlying mechanisms.

To distinguish effects of platelets and RBCs, we formed thrombin-induced clots from reconstituted human blood samples containing platelet-free plasma and washed platelet-free RBCs, followed by tracking the clot size. The structure and composition of the clots before and after RBC-induced shrinkage were analyzed using histology and scanning electron microscopy (SEM). Horizontal plate rheometer was used to measure the tension developed in the RBC-containing platelet-free plasma clots. The role of plasma proteins and osmotically active dextran in the RBC-induced clot shrinkage was examined using purified human fibrin(ogen).

Platelet-free clots formed in the presence of RBCs with 40% hematocrit exhibited spontaneous shrinkage of up to 30% of the initial clot volume within one hour. This process was insensitive to the inhibitors of platelet contractility, such as blebbistatin, latrunculin A, and abciximab. The RBC-induced clot shrinkage was augmented with an increase in RBC count from $4*10^6/\mu l$ to $6*10^6/\mu l$, whereas in the absence of RBCs no plasma clot shrinkage was observed. The results obtained clearly show that blood clot shrinkage can be caused by RBCs alone and that this effect, unlike the platelet-driven clot contraction, is unrelated to the intracellular contractile actomyosin machinery associated with the $\alpha IIb\beta 3$-mediated transmission of the traction force to the extracellular fibrin network.

Histology and SEM revealed that RBCs inside the shrunken clots formed aggregates, either as linear stacks (rouleaux) or unstructured three-dimensional accumulations. The intercellular adhesion of RBCs within the compacted fibrin clots suggests that the RBC aggregation is the driving force of clot shrinkage in the absence of platelets.

To quantify the tensile force generated by aggregating RBCs within a clot, dynamic rheometry of platelet-free blood clots during formation was performed. The average maximal bulk tensile force normalized by the total number of RBCs in the sample volume yielded an average force per one RBC of ~120±100 pN. This is an estimate of the tensile force generated by an aggregating RBC within a clot, which drives compaction of the fibrin network.

To assess the role of plasma proteins in the RBC-induced clot shrinkage and to understand the mechanisms of RBC aggregation within a clot, we formed thrombin-induced clots from purified fibrinogen (2 mg/ml) in the presence of RBCs ($4*10^6/\mu l$). In the absence of plasma proteins, the RBC-containing clots made of pure fibrin did not undergo shrinkage, indicating the necessity of plasma proteins for RBC aggregation and subsequent clot shrinkage. The current view on the mechanisms of plasma protein-induced RBC aggregation is based on the physical bridging and the osmotic depletion models. Notably, as fibrinogen is converted to fibrin, fibrinogen-mediated RBC bridging in a clot is unlikely, emphasizing the prevalence of the depletion mechanism. To confirm the importance of the depletion mechanism of RBC aggregation as a driving force of fibrin clot shrinkage, we added 100-kDa dextran (1-40 mg/ml) to purified fibrinogen mixed with RBCs before clotting with thrombin. In the presence of dextran, clot shrinkage was observed with a dose-dependent increase in final volume reduction. These results strongly suggest that the osmotic depletion interactions are the driving force of the RBC-induced fibrin clot shrinkage.

This study provides compelling evidence that RBCs play a role in blood clot shrinkage unrelated to platelets, suggesting that aggregating RBCs contribute actively to the blood clot contraction. Physiologically, the RBC-induced clot shrinkage may reinforce the platelet-driven blood clot contraction and/or promote clot compaction when there are few or dysfunctional platelets.

Disclosures No relevant conflicts of interest to declare.

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