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Transmission electron microscopy reveals ultrastructural differences between reticulated and non-reticulated human platelets

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Reticulated platelets (RP) are the youngest circulating platelets in blood. Compared to older platelets, RP represent a highly active prothrombotic platelet population associated with an increased risk for cardiovascular events, mortality and impaired response to antiplatelet drugs compared to older platelets (non-RP). The underlying mechanisms for these characteristics of RP are so far poorly understood.

This study aimed to characterize ultrastructural properties of RP and non-RP by transmission electron microscopy (TEM) of FACS-sorted human platelets using a novel staining method for RP.

Washed platelets from three healthy donors were stained by SYTO™13, a nucleic acid binding fluorescent dye, which enables determination of RP and non-RP based on their RNA-content. 8×10^6 platelets were fixed, sorted and sandwiched between two layers of agarose gel. Samples were further processed for visualization by TEM. In total, 1047 platelets, i.e., electron micrographs of individual cross-sections, were analysed by an investigator blinded concerning experimental condition. Sizes, numbers of α-granules, dense granules, mitochondria and open canalicular system openings were assessed in RP and non-RP, respectively. Furthermore, platelets were screened for pseudopodia formation as an indicator for activation.

Cross-sectional area was significantly different between RP and non-RP (2.44 [1.80–3.22] vs. 1.34 [1.04–1.89] μm^2; $p<0.0001$; median with IQR). α-granule and mitochondria amounts were higher in RP which persisted even after adjustment for platelet size (α-granules: 4.64 [3.46–5.86]/μm^2 vs. 4.15 [2.87–5.26]/μm^2; $p<0.0001$; mitochondria: 0.33±0.02 /μm^2 vs. 0.12±0.01/μm^2; mean ± SEM). In contrast, the amount of open canalicular system openings per square μm was higher in the non-RP group (5.82 [4.34–7.68] /μm^2 vs. 5.52 [4.01–7.11] /μm^2; p=0.009). Dense granule content per square μm was similar in both RP and non-RP. Pseudopodia were present in 38% (RP) respective 37% (non-RP) of platelets. Notably, golgi apparatus and rough endoplasmic reticulum which are rarely seen in platelets were detected in several RP.

Analysis of TEM pictures revealed an almost 2-fold higher cross-sectional area in RP compared to non-RP. Even after adjustment for differences in size, α-granule content remained significantly higher in RP indicating a higher storage pool for prothrombotic constituents like p-selectin or von Willebrand factor. Although the relative amount of dense granules per area did not differ between the two groups, a higher absolute number of dense granules per platelet in the RP group is indicative for higher amounts of stored small molecules such as ADP, calcium or serotonin. Despite the anucleate nature of platelets, the presence of golgi apparatus and rough endoplasmic reticulum suggests the capability of protein biosynthesis in RP. These comprehensive findings provide new important insight into the ultrastructural properties of human RP.