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The Arrangement of Microtubules in the Platelet Marginal Band

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During thrombosis, platelets must undergo a drastic change in shape over a short period of time [1]. In their inactive state they are discoid, after activation, platelets become a tight circular shape and then flatten out to form larger aggregations [1]. Overall, platelet cell shape is regulated by two opposing forces generated by the cytoskeleton: 1) cortical tension generated by the cortical actin and 2) the rigidity of the circular band of microtubules known as the marginal band [2]. During this transition, the cell reduces in size and the rigid marginal band of microtubules coil [2].

Although it is not as fast as the coiling of the marginal band, the microtubules in the platelet can also polymerize or depolymerize to increase or decrease their length. When examining the structure of the microtubule, the ends can be described as having a blunt, sheet, frayed, flared, or curled patterns [3]. The frayed, flared, and sheet appearance may indicate polymerization of microtubules as long as the blunt end is not capped. The curled tip pattern can suggest depolymerization of the microtubules. Eventually, the microtubules will depolymerize and the marginal band will disassemble, leading to flattened platelets with extended filopodia.

In this study, we used cryo-electron tomography (cryo-ET) to investigate changes to cytoskeleton ultrastructure in both inactive and activated platelets. Our goal was to determine the 1) spatial orientation of the microtubules, including the arrangement of the microtubule tips, 2) length of the microtubules, and 3) classify the types of microtubule tips present in platelets. Fresh platelets were isolated from blood and was used immediately to prepare cryo-grids with either a ThermoFisher Vitrobot or Gatan CP3 plunging system. Cryo-grids were imaged with either a 200 kV or 300 kV cryo-TEM (equipped with an energy filter and direct electron detector) to screen samples and collect tomography data. Tilt-series were acquired with SerialEM [4]. Tomographic reconstructions were generated using the IMOD software platform [5] and rendered and annotated with the Amira software package (ThermoFisher).

We found that the platelet microtubules are arranged in a marginal band and that the tips tend to form clusters. On average, the length of the microtubules is shorter in platelets that do not have a complete marginal band. Platelets tend to have more sheet-type microtubule tips, than are present in yeast and other human cell types, indicating that there is likely to be a high degree of microtubule reorganization [3]. Cryo-ET is an excellent technique for investigating the platelet cytoskeleton. In the future, we would like to investigate other proteins associated with the cytoskeleton [6].

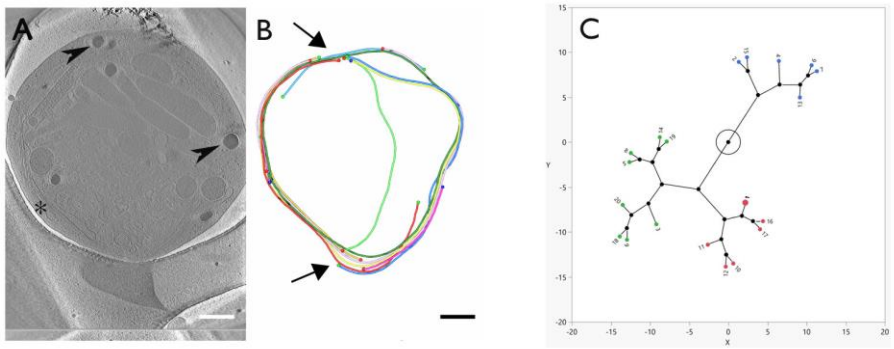

Figure 1. Arrangement of microtubules in platelets. A) Tomographic slice (18.52 nm thick) of a mouse platelet. The platelet is 3.8 m in diameter, has granules (arrowheads), and the marginal band (asterisk). B) A segmentation of the microtubules located in the marginal band of the platelet cell in (A), with clusters of microtubule tips (arrows). The microtubule marginal band is composed of multiple overlapping microtubules that are shorter than the circumference of the entire band. C) Cluster plot, showing the relative distance between the tips of the microtubules. Scale bars are 500 nm.

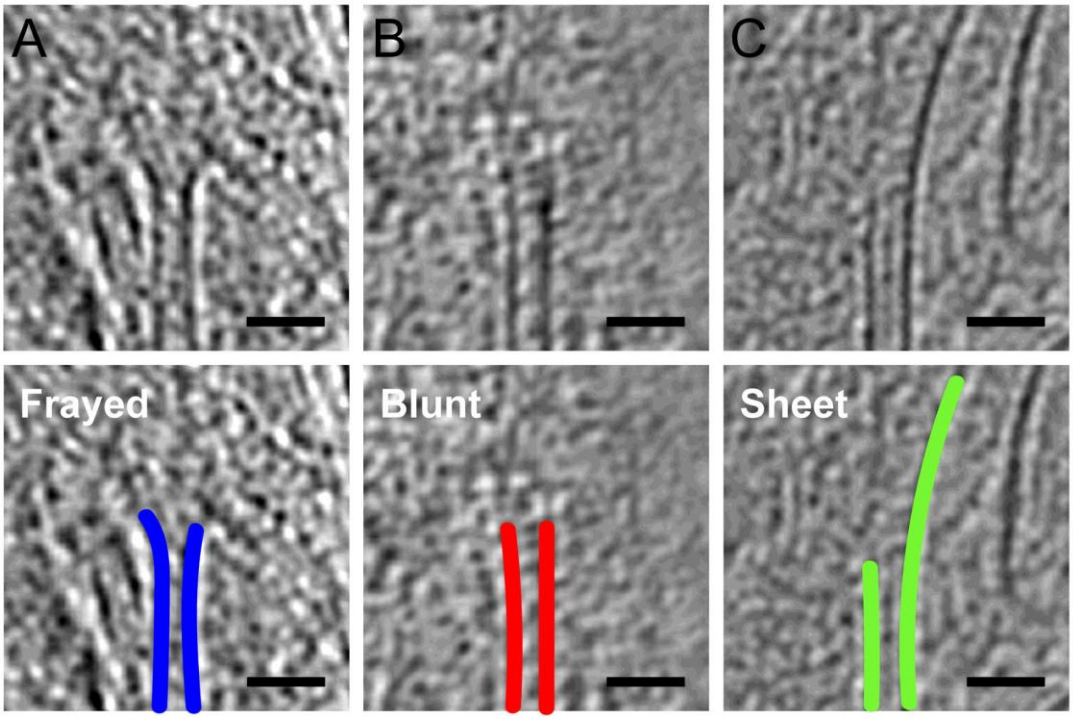

Figure 2. Tomographic slices and the matching rendered views of the microtubule tip types present in the marginal band of platelets A) Frayed, B) Blunt, and C) Sheet. Scale bars are 50 nm.

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