Human platelets isolated by apheresis are able to sequester bacteria by aggregation and to engulf them into the open canalicular system

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In order to avoid platelet (PLT) aggregation, PLT concentrates (PC) produced by thrombapheresis are stored at room temperature under soft agitation. Under this condition, the storage time is limited to few days since PLT are usually suspended in plasma or in plasma containing additive solution, providing an excellent growth environment for bacteria in the case of an occasional bacterial contamination. Transfusion of contaminated PC can cause septic transfusion reactions in the recipient ranging from mild to fatal and life threatening complications.

In a review, published by Fitzgerald et al. [1], several attachment sites and cell surface receptors on both, bacteria and PLT are described which are able to provoke PLT aggregation. In order to find out, whether bacteria can be attached to or engulfed by aggregated platelets, we investigated PC spiked with bacteria using transmission electron microscopy (TEM) and electron tomography (ET).

PLT obtained from apheresis were spiked with cultured and logarithmically growing bacteria (Staphylococcus epidermidis or Escherichia coli) in a ratio 1:1 for 1 hour. This suspension was prefixed with 1% formaldehyde freshly prepared from paraformaldehyde for 5 min, encapsulated in alginate beads and postfixed with 2.5% glutaraldehyde for 1 hour and in 1% buffered OsO_4 for 90 min. The alginate beads were dehydrated in a gradual series of ethanol and embedded in Spurr's epoxy resin. Ultrathin sections (70-80 nm) and semithin sections (250 nm) were cut using a diamond knife for TEM and ET respectively, to study the interaction between platelets and bacteria. ET was performed using a 200 KV TEM (Tecnai 20, FEI). Images from tilt series of 250 nm in the range of -70° to +70° with an increment of 1° were acquired. A software-(Explore 3D, FEI) triggered goniometer allowed to compensate dislocations during tilting. 3D-volumes were reconstructed by the help of the Inspect 3D software (FEI) and 3D-models were drawn using the AMIRA 4.1 (Mercury Computer Systems GmbH) software.

We could demonstrate that PLT are able to attach and ingest bacteria which are deposed in the open canalicular system (OCS) of PLT. Using ET, we could visualize that the OCS of aggregated PLT forms wide vesicular dilatations connected to each other by thin bridges (Figure 1). The activated and partially degranulated PLT extend filopodia forming aggregates by free cell contacts surrounding and sequestering bacteria. In addition, bacteria are also engulfed by the dilatated OCS (Figure 3). These results agree with those of Li et al [2] showing the engulfment of Porphyromonas gingivalis by PLT aggregates. The three

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dimensional structure of this system and also the interdigitating filopodia of aggregated PLT could be demonstrated for the first time by ET.

1. J.R. Fitzgerald et al., Nat Rev Microbiol **4** (2006) p445
2. X. Li et al. Thromb Res 122 (2008) p810

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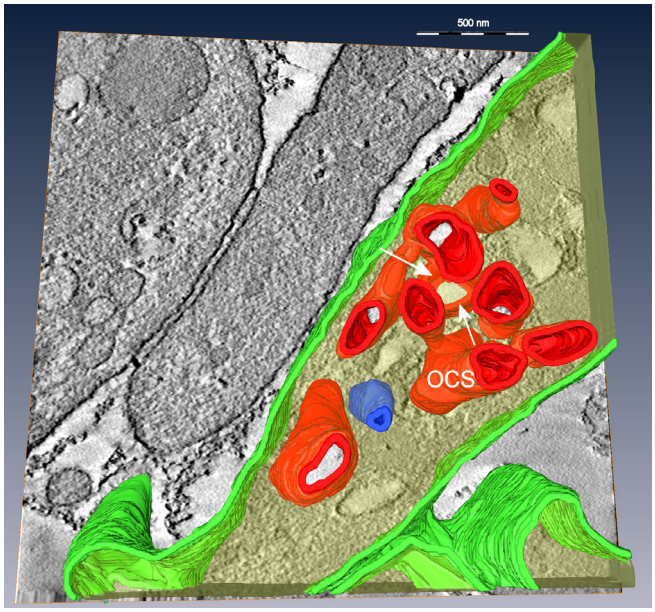

Figure 1. 3D model of aggregated PLT drawn after ET showing the vesicular dilatations of the OCS connected each other by small rims (arrows). Bar = 1 µm.

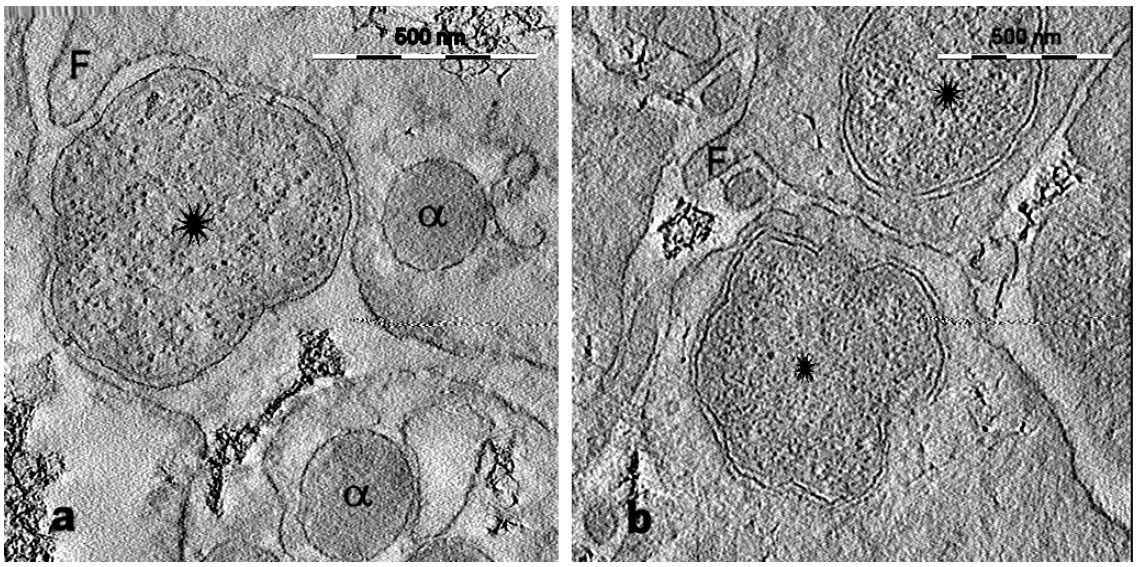

Figure 2. Sequestration of bacteria by PLT aggregates (**2a**) or by uptake into the OCS (**2b**). Virtual slices were taken from reconstructed volume. E. coli are marked by a star. F=filopodia, α=alpha granules.

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