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## *Enrichment of tight junction-like structures at sites of platelet-platelet contact*

Magdolna Nagy[1], Markus Bender[2], Natalie S. Poulter[3,4], Albert Sickmann[5], Xavier Stéphenne[6], Sanne L.N. Brouns[1], Rory R. Koenen[1], Hugo ten Cate[1,7,8,9], Johan W.M. Heemskerk[1,10], Constance C.F.M.J. Baaten[1,11]

[1]Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands
[2]Institute of Experimental Biomedicine – Chair I, University Hospital and Rudolf Virchow Center, Würzburg, Germany
[3]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom
[4]Centre of Membrane Proteins and Receptors (COMPARE), Universities of Birmingham and Nottingham, Midlands, United Kingdom
[5]Department of Protein dynamics, Leibniz Institute for Analytical Sciences - ISAS-e.V, Dortmund, Germany
[6]Laboratoire d'Hépatologie Pédiatrique et Thérapie Cellulaire, Unité PEDI, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain (UCLouvain), Brussels, Belgium
[7]Department of Internal Medicine, Maastricht University Medical Centre+, Maastricht, The Netherlands
[8]Thrombosis Expertise Centre, Maastricht University Medical Centre+, Maastricht, The Netherlands
[9]Center for Thrombosis and Haemostasis (CTH), Gutenberg University Medical Center, Mainz, Germany
[10]Synapse Research Institute, Maastricht, the Netherlands
[11]Institute for Molecular Cardiovascular Research (IMCAR), University Hospital RWTH Aachen, Aachen, Germany.

*Background:* In tissues, cell-cell adhesion, barrier formation and communication are regulated by junction proteins. Interestingly, platelets express several of these proteins too. Previously, research has shown that gap junction proteins expressed by platelets form gap junctions within platelet thrombi that are necessary to control the contraction of the clots. Also, platelet activation processes are negatively regulated by endothelial cell-selective adhesion molecule (ESAM) and junctional adhesion molecule A (JAM-A), proteins that are part of the tight junctions (TJ) of endothelial cells. However, how other components of TJs -like zonula occludens 2 (ZO-2)- concentrate at platelet-platelet contacts and influence these, has not yet been described.
*Methods:* Using transcriptomics and (phospho)proteomics databases, the expression and regulation upon platelet activation or inhibition of known tight junction proteins in platelets was investigated. Isolated human platelets were allowed to fully spread on a fibrinogen or laminin surface and were additionally activated by stimulating $P2Y_{12}$, PAR1 or GPVI signaling. Fixed samples were stained to assess F-actin polymerization in relation to CD61, ZO-2 and ESAM distribution using confocal and/or super-resolution fluorescence microscopy. Platinum replica electron microscopy was applied for ultrastructural visualization of the cytoskeleton at sites of platelet-platelet interaction.
*Results:* Confocal and super-resolution fluorescence microscopy indicated a marked redistribution and clustering of ZO-2 molecules at sites of platelet-platelet contacts, in which the colocalization of ZO-2 and ESAM was enhanced when platelets were additionally activated. Furthermore, platinum replica electron microscopy revealed that inter-platelet contacts resulted in the merging of the circumferential actin bundles between interacting platelets. These phenomena were antagonized by cAMP elevation.
*Conclusion:* Jointly, these data point to the assembly of tight junction-like structures, where intra- and extracellular components of tight junctions accumulate at sites of close platelet-platelet contact.

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