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Three-Dimensional Organization of Tight Junctions Investigated by TEM and STEM Tomography

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Endothelial cells provide a crucial interface between the blood and tissue environments. One of the important functions of endothelial cells in a blood vessels is to separate blood from underlying tissues. These cells function as gatekeepers, controlling the infiltration of blood proteins and cells into the vessel wall. This unique characteristic is achieved through specialized transcellular systems of transport vesicles and by the coordinated opening and closure of cell–cell junctions [1].

Endothelial cells have several types of specialized junctional regions that are comparable to adherens junctions (AJs) and tight junctions (TJs). Endothelial cells express cell type-specific transmembrane adhesion proteins, such as VE-cadherin at AJs [2] and claudin-5 at TJs [3]. The restricted cell specificity of these components indicates that they might be needed for selective cell–cell recognition and/or specific functional properties of endothelial cells. Some junctional proteins function as scaffolds, binding several effector proteins and facilitating their reciprocal interaction. A typical example is the TJ component: zona occludens-1 (ZO1), which associates with many transmembrane proteins, such as claudins, occludin or junctional adhesion molecules (JAMs) [4].

Using TEM and STEM tomography, we focus on the 3D architecture of the tight junction between endothelial cells in Human Umbilical Vein Endothelial Cells (HUVEC) cells Fig. 1. Electron tomography is the only technique that can provide 3D information with nm-scale resolution of unique samples. The first 3D structure of TJs of chemically fixed capillary tissue has been published in 1984 by Bundgaard [5]. However as it is known the chemically fixed material suffers from many artifacts and in addition no evidence of tight junctional proteins were presented. Therefore we employ an alternative method as high-pressure freezing followed by dehydration during freeze substitution and embedding in lowicryl resin. Additionally, pre-embedding labeling with ultra small gold of the tight functional proteins as claudin-5 or ZO-1 gives an unambiguous localization of the tight junctions. Furthermore, by combining TEM with HAADF-STEM imaging we aim at detecting ultra small (1.4nm) [6] gold labels within plastic-embedded sections to localized labeled molecules.

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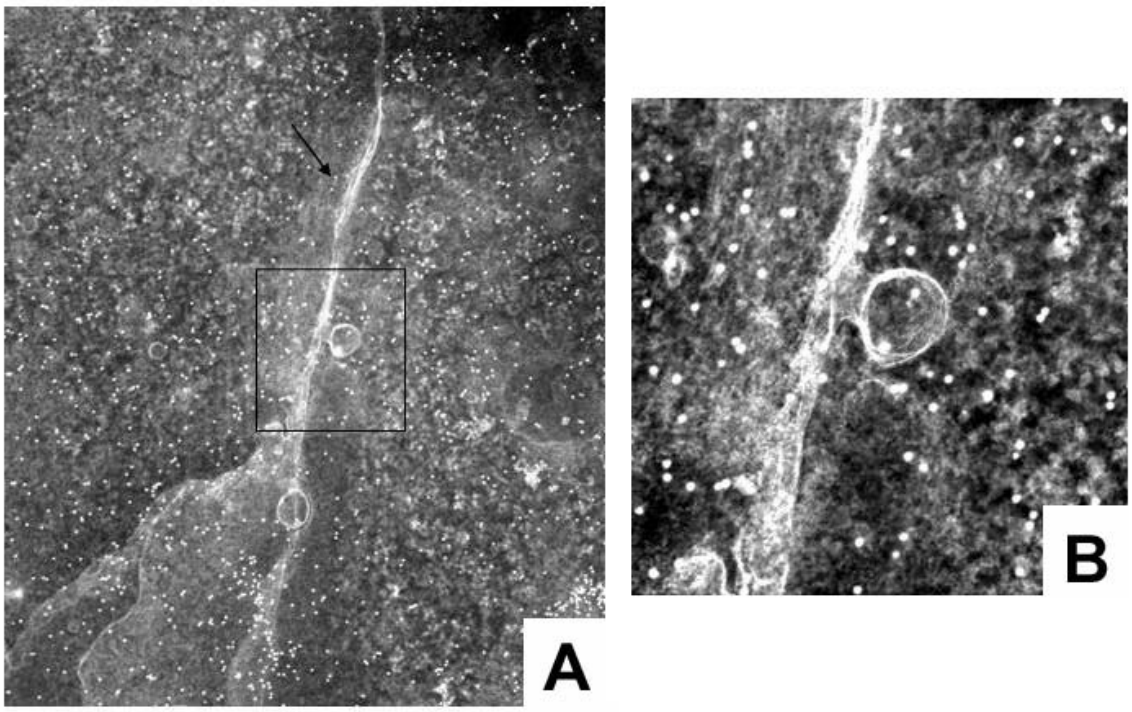

FIG 1. A. HAADF-STEM image of the junctional area in between HUVEC cells (indicated with an arrow). The black square indicates the presence of the caveolar invagination from the plasma membrane, which is enlarged in B. Using HAADF-STEM imaging the contrast of membranes and the 15 nm gold labels is strongly enhanced.

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