Ultrastructural & Analytical Methods in Life Sciences

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"Kinetic Studies" in Streptococcal Pathogenesis Applying Field Emission Scanning Electron Microscopy (FESEM)

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Electron microscopy has long been thought of as providing only a static picture. With time this kind of slight misconception has been changing gradually towards an approach which includes also some kinetic, dynamic data of processes following a triggered process. High resolution field emission electron microscopy (FESEM) is an important tool to study adherence and invasion mechanisms of pathogenic bacteria. As one deals with fixed samples a live-imaging is impossible. Nevertheless, with a consecutive FESEM imaging of a series of experiments one can gain insights into "kinetic mechanisms" of pathogenic bacteria. Here, "kinetic events" of adherence and invasion of GAS via integrin-clustering, intracellular trafficking and the exocytosis of certain serotypes of GAS will be discussed.

Group A Streptococci utilize the surface protein SfbI as a major fibronectin binding protein. Binding of fibronectin to the bacteria results in binding to $\alpha_5\beta_1$-integrins. It is known that integrins must cluster to trigger a signaling event. Binding of SfbI to gold-nanoparticles by van der Waals forces, which do not impair the biological function, allowed for the first time to visualize the kinetic of integrin-clustering on a cell surface of endothelial cells (HUVEC). Clustering of integrins subsequentely leads to a caveolae dependent invasion resulting in prevention of lysosomal fusion which was demonstrated by feeding host cells with BSA-coated gold-nanoparticle 12 h before infection. Streptococci have to pass the blood endothelial cell layer to gain access to deeper tissue. With BSA-coated gold-nanoparticle it was demonstrated that certain GAS hijack the exocytosis mechanism of host endothelial cells to pass over the blood endothelium to gain access to the underlying basal membrane and collagen.

Conclusion: High resolution field emission scanning electron microscopy together with gold-nanoparticle coated pathogenicity factors have been proven to be a potent tool to study even "kinetic mechanisms" of pathogenic bacteria.

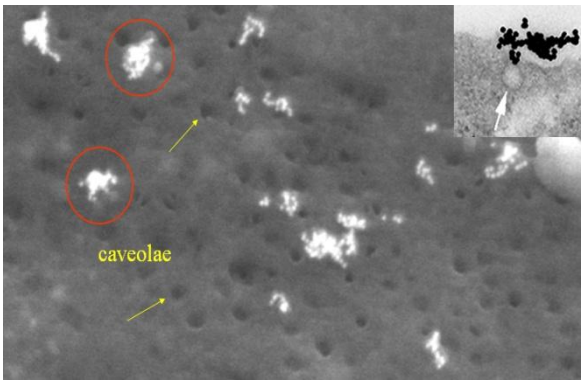

Figure 1.

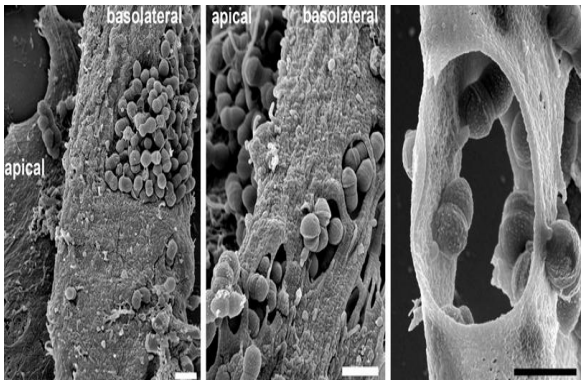

Figure 2.

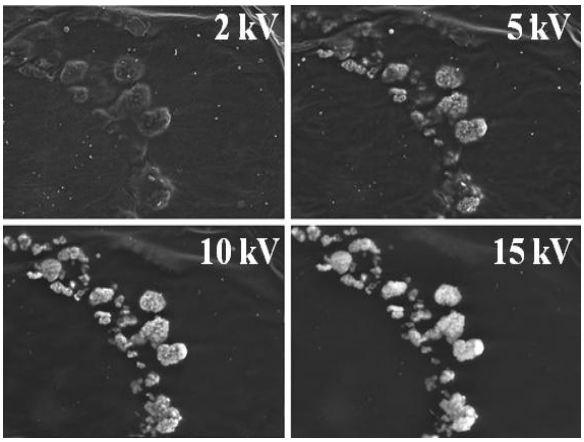

Figure 3.

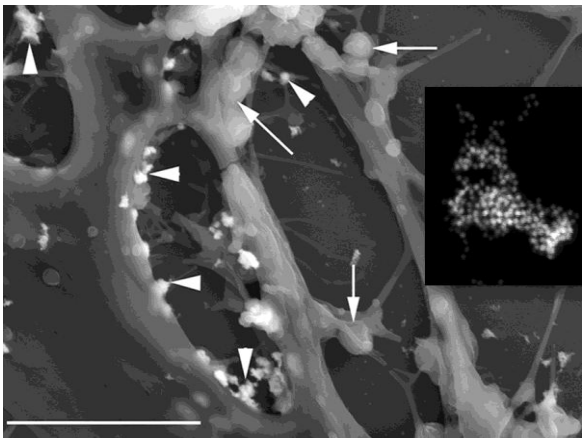

Figure 4.