From Emergency Diagnostics to Basic Research – Electron Microscopy in Medical Microbiology

Michael Laue

Advanced Light and Electron Microscopy, Center of Biological Threads and Special Pathogens 4 (ZBS 4), Robert Koch Institute, Nordufer 20, Berlin

Electron microscopy (EM) extends the optical resolution to the sub-nanometer range and therefore allows the analysis of small biological structures, such as viruses, bacteria, organelles or even larger molecules. In medical microbiology EM is widely used for various purposes. Diagnostic EM and the application of high-end imaging/preparation methods to answer questions of basic research are two distinct facets from the spectrum of EM applications that we use at the Robert Koch Institute (RKI).

A rapid diagnosis of a pathogen is necessary if a life-threatening communicable disease affects single persons or already spreads within the population. EM is one of the methods which are applied in these situations at the RKI. It complements light microscopy as a front-line method to screen samples for pathogens and allows detecting all particulate constituents of a sample down to the resolution limit, which is far below the size of the smallest known microorganisms including viruses. Diagnosis is rapid, usually completed well below one hour for single or few samples, but not very specific and therefore provides a rapid orientation that guides and speeds up the more specific methods (e.g. molecular genetics). Moreover, it serves as an independent generic control for results obtained by other methods involved and it visualizes the infectious unit. Standard preparation technique is the negative-staining technique which adsorbs particles of a sample suspension on a support and contrast them with a heavy-metal stain [1]. The whole preparation is simple, robust and takes only about a couple of minutes. Compact samples, which cannot be transferred into a suspension, can be prepared by ultra-rapid thin section techniques, which make these samples accessible for a diagnosis [1]. Current development of the methods include attempts to improve specificity of the diagnosis, e.g. by combining EM with spectroscopy techniques [2, 3].

Bacteria of the genera *Bacillus* and *Clostridium* may cause diseases, such as anthrax or wound infections. A particular feature of these bacteria is the ability to survive unpleasant environmental conditions by forming spores which are extremely resistant and which retain germination competence for years. The molecular and structural basis for this resistance is not fully understood. To unravel the architecture and chemical composition of bacterial spores we applied various high-end EM methods, such as high-pressure freezing, cryo-EM of vitreous sections and serial cryo-focussed ion-beam scanning-EM. We identified a couple of hitherto unknown structures in the dormant spore and analysed them in more detail, which also included following their fate during germination and outgrowth. For instance, the DNA seems to be organized in a crystal-like structure, which dissolves into strands during early germination. This structural change timely corresponds with the release of calcium and swelling of the core which is accompanied by the loss of heat resistance. Combining physiological parameters with structural and chemical changes allows us to provide new hypothesis on the mechanisms of the extraordinary resistance of spores and their rapid revitalisation during germination.

References

[1] M. Laue, *Meth Cell Biol.* 96, 1-20 (2010).
[2] M. Laue, G. Fulda, *J Microbiol Meth.* 94, 13-21 (2013).
[3] P. Hermann, A. Hermelink, V. Lausch, L. Möller, N. Bannert, D. Naumann, *Analyst* 136, 1148-1152 (2011).