Focused ion beam (FIB) / scanning electron microscopy (SEM) of epithelial tissue

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The SEM is a tool for visualizing the sample surface. A welcome characteristic of a SEM is its versatility in the accessories that could be installed in its specimen camber, such as, for instance, additional beams. When the FIB is incorporated, a system is called FIB/SEM system.

Focused ion beams have been used since the 1960s to investigate the chemical and isotopic composition of minerals. A focused ion beam blasts atoms and molecules free from the surface of a material, some of these free particles are also ions, and these are guided by electric fields to a mass spectrometer which identifies them with great precision. Because of the sputtering capability, the FIB is used also as a micro-machining tool to modify or machine materials at the micro and nano-scale.

Today's capabilities of FIB have proven to be applicable for a range of disciplines from materials to biological sciences. FIB/SEM is popular for advanced circuit edit, and for revealing below-the-surface defects in advanced materials and devices, for site-specific 2D sectioning and imaging of microstructures being of material or biological origin. A promising application of FIB/SEM for biological samples is the *in situ* site-specific manipulation of a specimen in order to expose subsurface structures for microscopy or elemental analyses (Drobne et al. 2008, Lešer et al. 2009). The FIB/SEM investigation can be applied on bulk biological samples, prepared for conventional SEM or on bulk resin-embedded specimens prepared for conventional TEM, what is at present more widespread.

In the work presented here, applicability of FIB/SEM in structural research of epithelial tissue prepared for conventional SEM was studied. In general, epithelial tissue is specialized to form the covering or lining of all internal and external body surfaces. Epithelial cells are subjected to a variety of stressors like mechanical injuries, chemicals, radiation, infections etc. Structural changes of epithelia often reveal the physiological state of an organism, its health status, side effects of drugs, toxic effects of chemicals etc. in vertebrates and also in invertebrates.

A biological specimen, contrary to materials normally requires fixation to preserve its structure. In SEM the outermost structures are examined, therefore they should be fixed properly. However, FIB milling demands also satisfactory fixation of subsurface structures. In the work presented here digestive glands of a terrestrial isopods, *Porcellio scaber* Latreille, 1809 (Crustacea: Isopoda), were selected as a model tissue. The best results in terms of topographical contrast were obtained with the aldehyde-fixed, osmium postfixation and conductively stained by TOTO (thiocarbohydrazide / osmium tetroxide / thiocarbohydrazide / osmium tetroxide; Fig. 1 a-d). This treatment provided the best ratio between the extracted and preserved material and gives enough relief to allow the distinction among different

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intracellular structures (Lešer et al. 2009). In our study, the rough FIB milling employed ion beam currents of 5 to 7 nA, at 30 kV. Lower beam currents of 100 to 300 pA were used to polish the cross section (FEI Strata DB 235 M, University of Modena, Italy). Spot size in the case of rough milling was approximately 150–100 nm of diameter, and for polishing it ranged from 20 nm to 35 nm of diameter. In biological samples, either trenches or cross sections are milled. In both cases the final goal of FIB manipulation is to expose subsurface structures for microscopy or analysis.

We conclude that, FIB/SEM enables simultaneous investigation of sample gross morphology, cell surface characteristics and subsurface structures. Besides, it allows *in situ* manipulation of a specimen (Fig. 1 a-d). Similar preparation procedure such as for conventional SEM could be used also for FIB/SEM. When the priority is put on the topographical contrast, best results are obtained with aldehyde-fixed and OTOTO processed samples. All these facts place the FIB/SEM in the arsenal of tools for structural research in biology.

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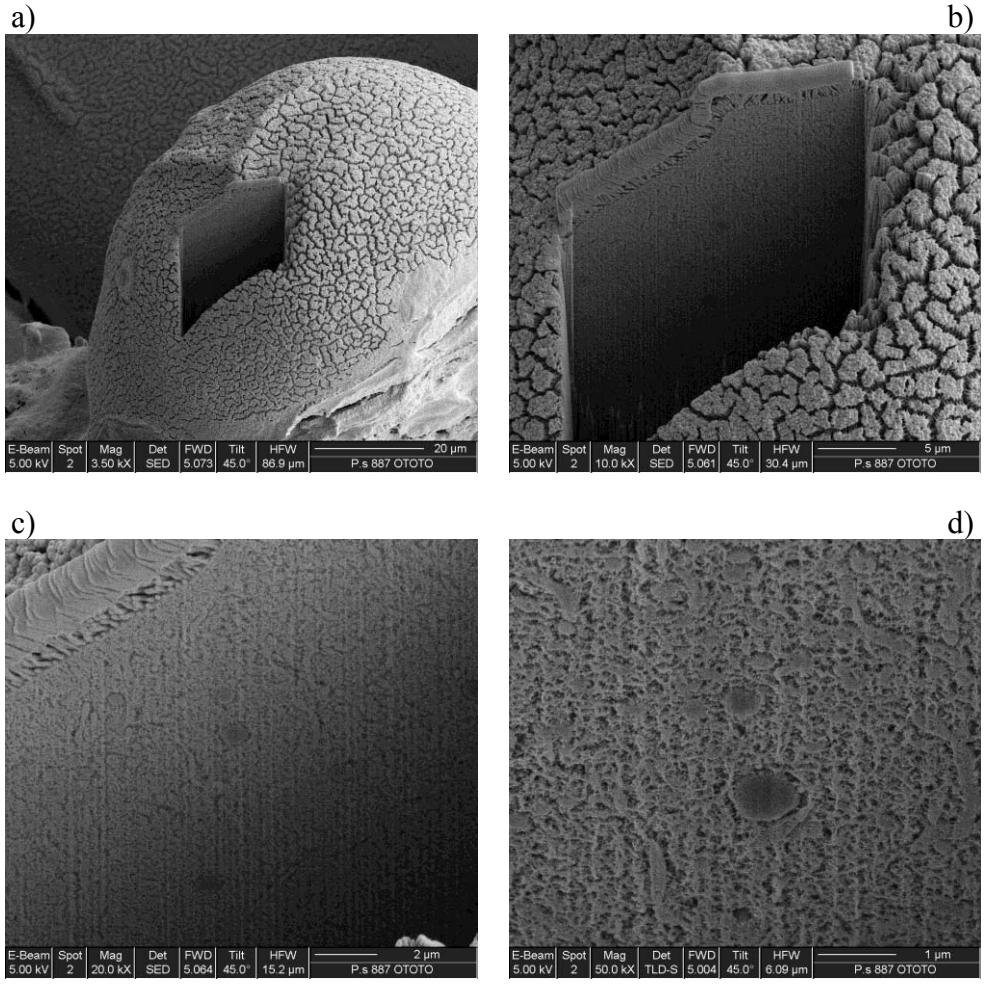

Figs 1 a-d. Electron micrographs of FIB/SEM investigated aldehyde primary fixed, osmium postfixed and conductively stained sample (TOTO - thiocarbohydrazide / osmium tetroxide / thiocarbohydrazide / osmium tetroxide). a-b) FIB milled trench on the apical part of digestive gland cell, c-d) lateral surface of the milled trench at different magnifications. Note platinum layer on the top of mill region which protects the upper sample surface (b, c).

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