SEMT

Society of Electron Microscope Technology

ONE DAY MEETING

Wednesday 31st March 2004

The School of Pharmacy
University Of London
Brunswick Square
London WC1N 1AX

The Society of Electron Microscope Technology

Prospective members should obtain an application form from the Hon. Secretary
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FUTURE PROGRAMME:

JUNE 16th 2004: VISIT TO FEI/PHILIPS RESEARCH LABS BRISTOL

OCTOBER 27th 2004: HALF DAY MEETING AT THE SCHOOL OF PHARMACY

The Society of Electron Microscope Technology

Programme

09.15-10.10	**Registration.**
10.10-10.15	**Introduction:** Chair, Heather Davies.
10.15-10.50	**Virus assembly in the endocytic pathway.** Dr. Annegret Pelchen-Matthews. MRC-LMCB, UCL London.
10.50-11.25	**Women defenceless against osteoporosis - can scanning electron microscopy help?** Dr. John Paul Cassela, Biological Forensic Pharmaceutical Sciences, Derby.
11.25-11.55	**Coffee.**
11.55-12.30	**Cell walls appear to swell during preparation methods for TEM when compared with cryo-SEM.** Kim Findlay, John Innes Centre, Norwich.
12.30-13.05	**Use of quantitative electron microscopy and 3-Dimensional reconstruction to study the effects of stress on synaptic and dendritic structures in mammalian hippocampus.** Prof. Michael Stewart, Biological Sciences dept., Open University.
13.05-14.30	**Lunch and Trade Exhibition.**
14.30-15.05	**AFM for cell biologists - integrating confocal and probe microscopy.** Prof. Mike Horton, The London Centre for Nanotechnology, UCL, London.
15.05-15.40	**Electron Microscopy and Breast Pathology : the search for "Myodifferentiation".** Dr Jorge Reis-Filho, Cancer Research UK, London.
15.40-16.00	**Tea.**
16.00-16.35	**Ice is Nice : Thin films of proteins and biomolecular assemblies imaged in amorphous ice.** Dr. Frank Booy, Biochemistry Dept. Imperial College London.
16.35-17.10	**The use of Analytical Scanning Electron Microscopy (SEM) in the investigation of damage to spacecraft.** Dr. Anton Kearsley, Natural History Museum, London.
17.10-18.30	**Wine Reception followed by a Conference Dinner.**

USE OF QUANTITATIVE ELECTRON MICROSCOPY AND 3-DIMENSIONAL RECONSTRUCTION TO STUDY THE EFFECTS OF STRESS ON SYNAPTIC AND DENDRITIC STRUCTURES IN MAMMALIAN HIPPOCAMPUS

Prof. Michael G. Stewart,
Dept. of Biological Sciences,
Open University, Walton Hall,
Milton Keynes, MK7 6AA,

Exposure to chronic restraint stress (CRS) in rats induces raised corticosteroid levels and may result in both cognitive impairment and a variety of morphological changes in area CA3 of the hippocampus. Previously we used 2-D stereology based upon the Disector method (Sterio, D.C. (1984) J. Microsc. 134 (Pt 2):127-36), to show that 21 days of CRS (6h per day) in rats induces a loss of simple unperforated synapses on thorns in striatum lucidum of CA3 (Sandi et al, Europ. J. Neurosci, vol 17, 2447-2456; 2003).

Here, I will present more detailed research data using 3-dimensional (3D) reconstruction methods which describe how 21 days of chronic restraint stress affects dendritic and spine morphology, and synaptic structure in the hippocampus, and how this affect is alleviated by a spatial learning paradigm. 3D reconstructions were made from electron microscopic images of ultrathin serial sections of dendritic segments from hippocampal area CA3. A large series consisting of more than 100 serial sections were used for each of the 3D reconstructions of entire dendritic segments from each of the 4 experimental animal states:

(i) Undisturbed control rats,
(ii) Chronic restraint stressed rats,
(iii) Water maze-trained rats and
(iv) Water maze-trained rats following restraint stress.

Electron microscope negatives were scanned at 900d.pi and tracing, alignment and reconstructions made in part using software available at http://synapses.bu.edu./. Detailed quantitative analyses were made of thorny excrescences on the dendritic segments, and of the organization of postsynaptic densities (PSDs) on these structures. Thorny excrescences consist of a stalk which contains numerous simple spines or thorns covered by perforated and unperforated post synaptic densities (PSDs).

Following stress our 3D data show that there is a marked retraction of thorns compared to unrestrained (control rats). This process is substantially reversed 24h following water maze training. Water maze training alone induces an increase in the size and volume of thorns, in comparison to thorns of unrestrained (control) rats. In addition in water maze trained rats, there is an increase in endosome content, in particular, multivesicular bodies in thorns which is complimented by the formation of numerous coated pits and coated vesicles in both dendrites and thorns. Endosomes may participate in sorting membranous proteins for degradation and receptor recycling; hence change in their numbers might be correlated to alterations in the physiological state of the rats.

These data show the remarkable plasticity of area CA3 of the hippocampus, where the deleterious effects of stress can be rapidly reversed by a behavioural training task. They also provide a neuroanatomical basis for stress-suppressing and learning-inducing plasticity properties.

"Women defenceless against osteoporosis - can scanning electron microscopy help?"

Dr JP Cassella [1], S. Elliott [2] D.I. Walton [2]
Division of Biological Forensic and Pharmaceutical Sciences
Division of Earth System Sciences
School of Education, Health & Sciences
University of Derby

Millions of women at risk from developing osteoporosis face being left with no safe preventative treatment due to the HRT debacle. Currently 70, 000 suffer hip fractures and 14, 000 die as a result.

The Governments National Institute for Clinical Excellence (NICE) is expected to rule against the use of a class of drugs known as bisphosphonates as a preventative therapy to possibly replace HRT. However to do so would leave a generation of women with no effective preventative treatments and could lead to thousands more falling victim to osteoporosis which affects 1 in 3 women over 50.

Studies using a shell-model from the Glottidea family have demonstrated that the refusal of pamidronate therapy by NICE may be a wise decision.

Glottidea form calcium phosphate in their shells unlike many other shell creatures which form calcium carbonate. This makes Glottidea ideal for studies of the effects of drugs on mineral formation. Using Scanning Electron Microscopy and X-Ray microanalysis, studies have shown abnormal changes in the shell architecture and in the calcium-phosphate mineral which forms in these shells when treated with bisphosphonates. Should such changes occur in human bone, the results could more damaging than the osteoporosis.

If neither HRT nor pamidronate is suitable, then a more natural treatment may be available. Research using high doses of vegetables such as the onion and herbs such as sage have had promising results in both human and animal trials. SEM may be able to help demonstrate the positive effect on bone mineral formation in these treated animals.

Cell walls appear to swell during preparation methods for TEM when compared with cryo-SEM.

Kim Findlay, John Innes Centre, Norwich

Comparative analysis of cryo-SEM with chemically fixed material suggests that conventional preparation methods promote wall swelling in plant cells. Results showed that cell wall thickness measurements in the TEM were commonly double those obtained by cryo-SEM, even when compared to high pressure frozen samples.

In order to grow, plant cells must divide and expand. The tensile strength of the cell wall allows turgor pressure to develop, which drives cell expansion. During expansion, new material must be incorporated into the wall or it must thin to zero. This implies that wall biosynthesis and cell expansion are interdependent processes. Previously published data, using measurements of wall thickness taken from conventionally fixed material, showed that these processes are uncoupled since wall thickness remained constant or increased during growth. However, the accuracy of these observations is now in question since we have shown, using cryo-SEM, that cell wall thinning indeed occurs in elongating Arabidopsis hypocotyls.

"AFM for cell biologists - integrating confocal and probe microscopy."

Mike Horton, Dept. of Medicine and The London Centre for Nanotechnology, University College London [m.horton@ucl.ac.uk]

Since the 1980s, when Binnig invented the scanning tunnelling (STM) and atomic force (AFM) microscopes, attempts have been made to demonstrate their value in biology. However, derivative 'scanned probe' analytical techniques, usually based upon commercial sytems, have only just begun to gain acceptance into mainstream biological research. Current applications range from high resolution imaging and functional analysis of bio-molecules at the single molecule level to, more recently, investigation of living cells. We have adapted commercial AFMs and integrated them with standard optical systems to create a 'bio-AFM' system that has been successfully used in experimental cell biology. Our current instrumental set-up and examples of applications from our own work (and of others) will be reviewed.

CHARRAS, G., LEHENKARI, P. & HORTON, M. (2002). "Biotechnological applications of atomic force microscopy". Methods Cell Biol. 68, 171-191

Virus assembly in the endocytic pathway

Annegret Pelchen-Matthews, Beatrice Kramer, Alberto Fraile-Ramos and Mark Marsh,
Cell Biology Unit, MRC-LMCB, UCL, Gower Street, London WC1E 6BT, UK

Enveloped viruses acquire their membrane by budding through a cellular membrane. We have studied this process by immunogold labelling of ultrathin cryosections of infected cells, which allows visualisation of virus particles at high resolution. We have shown that for the human and simian immunodeficiency viruses (HIV-1 and SIV) virus budding occurs not only at the plasma membrane, but also into endocytic organelles. In infected primary macrophages, the majority of budding figures and mature virus particles are associated with late endosomes with the morphology of multi-vesicular bodies (MVB). Infectious virus can be recovered from the supernatants of these cells, indicating that intracellular viruses are released from cells when the MVB fuse with the plasma membrane. Similarly, human cytomegalovirus (HCMV) particles acquire their membranes from either MVB or from small membrane vesicles that may traffic to or from MVB. Together with other observations, these studies indicate that, for a number of enveloped viruses, the endocytic pathway plays an important role in co-ordinating viral assembly and release.

Electron Microscopy and Breast Pathology : the search for "Myodifferentiation."

Dr Jorge Reis-Filho, The Breakthrough Toby Robins Breast Cancer Research Centre
Institute of Cancer Research, Mary-Jean Mitchell Green Building
Chester Beatty Laboratories, Fulham Road, London SW3 6JB UK

Myoepithelial and 'basal' differentiation in breast carcinomas has received great attention over the last few years, owing to the highly publicised cDNA array studies that have shown that some breast carcinomas may have a non-luminal transcriptome. But how novel is this concept? In this lecture, we aim to demonstrate that basal / myoepithelial differentiation in breast carcinomas was actually described over 30 years ago in small studies and anecdotal case reports. Surprisingly, the idea of breast carcinomas with divergent basal / myoepithelial differentiation was not taken seriously at that time. Currently, tumours with basal/myoepithelial phenotype can be recognised at the morphological and immunohistochemical level and several lines of evidence suggest they may be distinct biological entities. In addition, issues concerning histogenesis and differentiation in breast carcinomas will be discussed and basal / myoepithelial salivary gland neoplasms will be used to illustrate how ultrastructure and immunohistochemistry can be used for the characterisation of these neoplasms.

Ice is Nice : Thin films of proteins and biomolecular assemblies imaged in amorphous ice.

Frank Booy, Biochemistry Department.
Wolfson Laboratory Imperial College Exhibition Road London SW7 2AZ

Negative staining remains a robust and reliable means of visualising proteins and biomolecular assemblies by electron microscopy. Nevertheless it does have its limitations resulting from strong stain - protein interactions, structural collapse and stain recrystallisation which limit the resolution.
Cryo-electron microscopy can overcome some of these limitations as will be illustrated by comparing cryo-EM and negative staining for phage T4 and influenza virus. When combined with image processing cryo-EM is a very powerful method of structural analysis that can rival X-ray crystallography in the best case, whilst requiring orders of magnitude less material. Even at modest resolution, especially when combined with other biochemical data, cryo-EM has proven very useful and has, for example, permitted the localisation of the major proteins in the nucleocapsids of herpes simplex virus. Other examples of cryo-EM will be presented to illustrate the wide applicability of the technique.

The use of Analytical Scanning Electron Microscopy (SEM) in the investigation of damage to spacecraft.

Anton Kearsley, Natural History Museum, London

My talk will cover the use of analytical scanning electron microscopy (SEM) in the investigation of damage to spacecraft. Shuttle orbiter missions have returned samples from a number of satellites in low Earth orbit, including the Long Duration Exposure Facility (LDEF) a spacecraft designed to monitor the space environment. However, the most extensive surveys have been carried out on samples of the huge solar arrays carried by the Hubble Space Telescope (HST), and returned to Earth following service missions in 1993 and 2002. Hypervelocity impacts by pieces of man-made space debris (moving at perhaps 10km per second) and micrometeoroids (from comets and asteroids, moving at between 20 and 70 km per second) blast holes out of the glass covered silicon cells that generate power for the telescope. Most of these high velocity vandals leave behind distinctive chemical traces within the craters that they have made. X-ray mapping of the surface of the solar cells provides a quick and reliable way to locate and analyse the remains of shattered and vapourised cosmic bullets. These data allow the space agencies and satellite manufacturers to design robust yet cost effective equipment to survive the hostile environment of space.

I will illustrate my talk with examples from the damage surveys that I have performed, with some explanation of how very modern SEM and energy dispersive X-ray spectrometers have made the task much easier. I'll concentrate on the recent shuttle mission by Columbia, immediately prior to the tragedy, but will also show examples from Japanese and Russian spacecraft too.