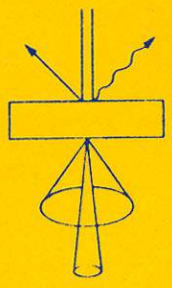
6-12-89

SEMT

Society of Electron Microscope Technology

Affiliated to the Royal Microscopical Society



Dr G M Lewis
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You are cordially invited to a meeting of the SEMT on Wednesday, December 6th. at the Imperial Cancer Research Fund, Lincolns Inn Fields, which incorporates the RMS Beginners' Competition.

Programme

1.45 Registration.

2.00 Chairman's Introduction.

2.05 "The past, present and future of Electron Microscopy"
Dr. Julian Beesley (Wellcoms Research Laboratories, Beckenham)

2.50 Tea

RMS Beginners' Competition

3.10 John P. Cassella (Inst. Dermatol., St. Thomas's Hosp. Med. School)
"Ultrastructural demonstration of skin antigens using silver-enhanced 1 nm gold probes".

3.25 Robert Scotland (Botany Dept., British Museum of Natural History)
"Homology, anatomy and microscopy"

3.40 Frances Wall (Dept. of Mineralogy, British Museum of Natural History)
"Breaking all the rules - the X-ray microanalysis of unprepared minerals"

3.55 Simon Hall (Dept. of Cell and Structural Biol, Univ. of Manchester)
"Elemental changes in cultured porcine endothelial cells after damage caused by the superoxide free radical".

4.10 John Spratt (Dept. of Mineralogy, British Museum of Natural History)
"Investigations into the coating of non-conductive specimens for high resolution SEM"

4.25 Desmond Tobin (Dept. Biochem., St. Thomas's Hospital Med. Sch.)
"Ultrastructural observations on the hair bulb melanocytes and melanosomes in acute alopecia areata".

4.40 Wine and mince pies, while the panel of Judges decide the winner of the £50 prize awarded by the RMS.

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Please complete this slip if you are able to attend and send it to the Secretary. Dr. Jill Lewis at the address above.

Name .....

Address .....

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I hope to attend the meeting on Dec. 6th.

S.E.M.T.

RMS Beginners' Competition, Dec. 6th., 1989. Abstracts.

1. "Ultrastructural demonstration of skin antigens using silver enhanced 1 nm gold probes"

John Paul Cassella (Dept. of Cell Pathol., Inst. of Dermatology, St. Thomas's Hosp. Med. Sch.)

Post embedding immunoelectron microscopy (IEM) has several advantages compared with pre-embedding IEM. However, the demonstration of sparsely distributed proteins, for example, skin basement membrane zone (BMZ) antigens, is difficult on the surface of post-embedded sections.
Due to the small number of epitopes of such proteins exposed on the surface of resin sections, the study of BMZ antigens using 5 nm immunogold conjugates has, to date, yielded little success. In order to demonstrate these antigens more clearly, a 1 nm immunogold conjugate has been utilized with silver enhancement on both semithin and ultrathin sections.
Fresh normal human skin (NHS) was cryofixed by plunging into liquid propane (-190 C) and cryosubstituted (-80 C) in 100% methanol. The skin was then embedded in Lowicryl K11M and polymerized (-60 C).
Semithin sections were cut and collected on glass slides. The sections were then incubated with the following primary antibodies: a polyclonal antibody against synthetic peptides of bullous pemphigoid antigen (BPAg), and a monoclonal antibody against the carboxy terminus of type VII collagen (c-VII-c). This was followed by incubation with a biotinylated secondary antibody. 1 nm gold conjugated avidin was then applied. Finally the sections were incubated in immunogold silver enhancement solution (the reaction being controlled microscopically), and examined under light microscopy.
Ultrathin sections were cut from the same NHS specimen and collected on nickel grids. These were immunolabelled and silver enhanced in the same way as semithin sections. The grids were then examined under transmission EM.
Electron and light microscopic analysis of the sections indicated the presence of BPAg on the hemidesmosomes and of c-VII-c in the lamina densa and sub lamina densa zone.
The use of 1 nm gold labelling with silver enhancement has allowed improved visualisation of antigens associated with the skin basement membrane zone at both light and electron microscopic levels. Thus it may be concluded that this improved immunolabelling technique allows simultaneous demonstration of sparsely distributed antigens with greater sensitivity than previous techniques involving larger gold particles.

2. "Homology, anatomy and microscopy"

Robert Scotland (Pollen Section, Botany Dept., British Museum of Natural History)

The concept of homology is one of the fundamental principles of biological systematics. Richard Owen, the first Director of the Natural History Museum, defined it as "structural correspondence under every variety of form and function". The opposite of homology is analogy, which Owen defined as "superficial or misleading similarity".
Character congruence (aggreement with other characters) and rigorous anatomical interpretation are essential components of homology. This will be illustrated by an example. I will show how scanning electron microscopy has helped in developing new character concepts of pollen morphology in the flowering plant family Acanthaceae.

3. "Breaking all the rules - the X-ray microanalysis of unprepared minerals.

Frances Wall (Dept. of Mineralogy, British Museum of Natural History)

A chemical analysis by electron microprobe is often an important part of mineral identification and characterization. Ideally, a sample of the mineral to be analysed would be taken to be polished and carbon coated but due to lack of time, small specimen size, or when dealing with objects d'art, this is not always possible. Neverthelss useful information may still be obtained by "breaking all the rules" and putting unprepared material into a scanning electron microscope equipped with a large specimen chamber, backscattered electron detector and energy-dispersive analysis system.
Fully quantitative chemical analysis and top quality high magnification photomicrographs are impossible but not necessarily needed. With a healthy respect for charging artifacts such as apparent depression of the accelerating voltage, sample movement, and stray X-rays and for surface problems such as topography, dirt and alteration it is possible to provide much useful data and solve many routine enquiries.

4. "Elemental changes in cultured porcine endothelial cells after damage caused by the superoxide free radical"

Simon Hall (Dept, of Cell and Structural Biol., Univ. of Manchester)

Electron probe microanalysis has been used to investigate the changes in concentrations of intracellular ions within endothelial cells cultured on microcarrier beads and treated with different concentrations of the xanthine oxidase/hypoxanthine reaction complex. Cells were washed (to remove extracellular ions), rapidly frozen on a Millipore filter membrane, then freeze dried, carbon coated and examined in a Cambridge 360 scanning electron microscope. Quantitative determination of elemental composition was carried out with reference to a cobalt standard.
Toxicological effects of the superoxide radical on the elemental composition of the endothelial cells were observed and compared to control (untreated) cells both in the short term (before gross ultrastructural changes became apparent) and over an extended six hour time course.
Preliminary studies have indicated the following mean elemental composition (in mol/Kg dry wt) for control cells - Na (787), Mg (33), P (152), S (128), Cl (1802) and K (20), with an average K/Na ratio of 0.03. Cells that have been damaged by the superoxide anion show an early increase in the level of Na, with a decreased K/Na ratio.
Changes in the elemental composition & fine structure of superoxide-treated cells will be considered in relation to the known chemical effects of the anion and the possible physiological changes that are taking place.

5. "Investigations into the coating of non-conductive specimens for high resolution scanning electron microscopy (HRSEM).

John Spratt (E.M.U., British Museum (Natural History)

The operating parameters of the scanning electron microscope (SEM) require that nearly all non-conducting specimens observed are coated with a thin metallic film. This improves electrical conductivity preventing surface electrical charge building up (charging), and also gives better thermal conductivity reducing damage caused by localized heating (beam damage). Secondary electron emission is also increased. Early specimen coating regimes relied upon evaporative coatings which were unsatisfactory due to large grain size and damage caused by heat to the speciment At present the most widely used method is sputter coating with gold or gold/palladium. Recent advances in SEM technology have lead to a steady improvement in resolution and at present is in the order of 0.7 nm. At this resolution coating regimes need to be reevaluated as it is now possible to resolve the coating and thus fine specimen detail is obscured.
An optimum coating for HRSEM is one which cannot be resolved but gives good electrical conductivity, thermal conductivity and secondary electron emission. Investigations into different metals and methods of coating are considered.

6. "Ultrastructural observations on the hair bulb melanocytes and melanosomes in alopecia areata.

Desmond Tobin (Unit of Cell Biol., Biochem. Dept., St. Thomas's Hosp. Med. Sch.)

It is well recognised that alopecia areata may preferentially affect pigmented hair, spares white hair, and that regrowing hair in the disease is often initially white. In addition there is an association with vitiligo and ocular depigmentation. To date the pathomechanisms of the melanocyte effects are unclear. We studied 10 patients with untreated acute alopecia areata, and 3 normal people with no clinically identifiable hair growth abnormality.
The morphology of the hair bulb melanocytes was studied using conventional light and electron microscopy. Morphologic changes were both cytoplasmic and nuclear; however, cytoplasmic alterations in the affected melanocytes often predated nuclear hyperchromatism. Also incomplete or "aborted" melanisation of the affected melanosomes were seen. Increased numbers of bizarre melanosomes were found in affected melanocytes compared with normal ones. Affected melanosomes had little pigment deposition, were disrupted, enlarged and rounded, no longer having their normal ellipsoid shape.
In several patients an unusual outer root sheath distribution of hair bulb melanocytes was seen . Atypical melanosome effects included marked pigment displacement into peribulbar and dermal papilla melanophages, with the matrix devoid of melanocytes or pigment. In the dermal papilla of some patients clumped pigment granules formed giant spherical melanin complexes without discernible limiting membranes, which, in some cases were close to lymphocytes. These morphological changes would appear to indicate an active attack on melanocytes, supporting the evidence for a melanocytic association in the etiology of alopecia areata.

Digital Storage and Archiving of SEM and TEM Images.

John Spratt

E.M.Unit, Dept. Mineralogy, British Museum (Natural History), Cromwell Rd, London. SW7 5BD.

Electron microscopes are used to examine an area or structure to produce an image which can be readily interpreted. The image produced is in two forms, a temporary or live image (screen) and a permanent image (photomicrograph). Producing photomicrographs for each image is costly in time and storage. Interpretation of the image is usually done from photomicrographs eg. measurements and comparisons, this can also be a time consuming process. An alternative to this is to digitise the image and use a computer to store and manipulate the image. In the last few years there has been a rapid increase in the numbers of commercial and home made image analysis and archiving systems available.

The E.M.Unit at the Natural History Museum has assembled such a system and has used it for such diverse purposes including, estimating the age at death of bones, measurement of spores and to produce a database of the units 35mm slide collection.