

6-12-91

SEMT

Society of Electron Microscope
Technology



Affiliated to the Royal Microscopical Society

ALTERNATIVES TO GOLD LABELLING

Friday, 6 December 1991

at

IMPERIAL CANCER RESEARCH FUND
Lincoln's Inn Fields, London WC2

PROGRAMME

2.00 Introduction - Pauline Barber

2.10 Ultrastructural antigen detection

Catherine Sarraf (Royal Postgraduate Medical School, London, W 12)

2.50 High resolution autoradiography

Mike Williams (Dept of Biomedical Science, University of Sheffield)

3.30 Autoradiography in the SEM

Cisele Hodges (Imperial Cancer Research Fund, London WC2)

3.50 Tea

4.10 Labelling the extracellular environment in a natural microbial flora

Hubert Newman (Dept of Periodontology, Eastman Dental Hospital)

4.40 General discussion followed by finger buffet with wine

To the Secretary:

Dr Jill Lewis, Electron Microscope Unit,
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I hope to attend the meeting at ICRF on 6 December 1991

Name Telephone

Address

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ABSTRACTS

ULTRASTRUCTURAL ANTIGEN DETECTION

Catherine Sarraf (Royal Postgraduate Medical School, London, W 12)

The problems of preservation of both antigenicity and morphology during tissue processing for EM are discussed. Although in pre-embedding techniques, the antibody is applied before antigenicity is lost through dehydration and embedding, there remains the problem of its penetration into the thick section and the drawback that no identical control tissue is available. Loss of antigenicity in post-embedding procedures can be minimised by careful choice of fixative (preferably perfused), treatment with sodium meta-periodate after osmication, rapid dehydration or use of hydrophilic resins. Sub-cellular localisation of antigens is specific and serial sections can be used as controls; multiple labelling is possible.

The advantages and difficulties of ultra-cryotomy are considered together with freeze etching and label fracture. Methods of immuno-labelling usually direct the primary antibody against the antigen of interest and a secondary antibody against the species in which the primary antibody was raised; these may have either enzymatic markers (in conjunction with diaminobenzidine) or particulate markers (e.g. ferritin or gold). Experiments will be described involving double labelling using both these techniques.

HIGH RESOLUTION AUTORADIOGRAPHY

or

'~~ALL~~ THAT GLISTERS IS NOT GOLD'

Mike Williams (Dept of Biomedical Science, University of Sheffield)

Autoradiography has developed steadily in refinement over a long period. During this time it has seen cytochemical methods and fashions come and go! That the approach is still in use in cell and tissue biology, toxicology, pathology and pharmacology indicates its continuing usefulness. Its particular value seems to reside in its power in two types of circumstance: first, its ability to give time-based (kinetic) data at sub-tissue and sub-cellular levels; second, its ability to permit a localising question to be asked even when no cytochemical staining method is available.

AUTORADIOGRAPHY IN THE SEM

Gisele Hodges (Imperial Cancer Research Fund, London, WC2)

A brief historical overview will be given and the following discussed:- the methodological basis for SEM autoradiography, quantitative evaluation of SEM autoradiograms by X-ray spectroscopy, and a survey of published applications.

References

Hodges G M and Muir M D (1974) Autoradiography of biological tissues in the SEM. *Nature* 247: 383-385

Hodges G M and Muir M D (1976) SEM Autoradiography. in: *Principles and Technology of Scanning Electron Microscopy*, Vol 5 (ed. M A Hayat), pp 78-93. Van Nostrand, New York.

Cheng G, Hodges G M and Trejdosiewicz L K (1985) A methodological basis for SEM Autoradiography: biosynthesis and radioligand binding. *J. Microscopy* 137: 9-16

Sanders C L, McDonald K E and Lauhala K E (1988) SEM Autoradiography: aggregation of inhaled PuO. *Int. J. Radiat. Biol.* 54: 115-121

LABELLING THE EXTRACELLULAR ENVIRONMENT IN A NATURAL MICROBIAL FLORA

Hubert Newman (Dept of Periodontology, Inst of Dental Surgery)

Dental plaque is part of the human natural microflora. It is the cause of the two commonest human diseases, chronic inflammatory periodontal disease (CIPD) and dental caries. This paradox of a natural flora causing widespread disease requires a detailed study of dental plaque ecology. This in turn is considerably helped by a close examination of that flora in its natural state.

Plaque forms on a cuticle, a residue of the organ that forms tooth enamel. Attachment between the two is mediated by a variety of polyanionic macromolecules, bacterial extracellular polysaccharides and host glycoproteins, from saliva and gingival fluid in particular. Using such procedures as colloidal iron, Alcian Blue-lanthanum nitrate and Ruthenium Red, the different types of adhesion of bacteria to cuticle and each other may be studied. The structural configurations may be confirmed by freeze-etching, to avoid artefacts due to dehydration.

As carbohydrate metabolism is crucial in dental caries and the early stages of chronic gingivitis, periodic acid-thiosemicarbazide/thiocarbohydrazide-silver proteinate may be of help to label intracellular polysaccharides, and enzyme labelling techniques may clarify such relevant features as metabolic environment. The combination of procedures, SEM, TEM, FEM for example, with electron histochemistry, may further serve to clarify the changes in plaque that result in disease. Thus the dental plaque at the stagnant risk site between teeth undergoes the following changes: host response factors kill colonising bacteria except those which are saccharolytic, acidogenic, and intracellular and extracellular polysaccharide producers.

In conclusion, it is unlikely that single procedures, even high technology EM, can tell us what we need to know about a given phenomenon. What is usually required is a combination of procedures, certainly often requiring in situ identification of cells, cell moieties or matrix elements. Electron histochemistry continues to have a role in these studies of the oral microbiota.