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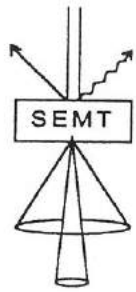

BEGINNERS' MEETING

Competition sponsored by the Royal Microscopical Society

WEDNESDAY 11 JUNE 1997

EASTMAN DENTAL HOSPITAL
Grays Inn Road
London WC1X 8LD

2.00 **Introduction** - The Chairman

2.05 **Microscopes and examination of Jurassic Parks**
Phil Wilby (British Geological Survey) - a past Competition winner

COMPETITION

2.45 **Microscopy of biofilms on intraocular lenses**
Louis McLaughlin-Borlace (Inst of Ophthalmology, London)

3.00 **Characterisation of two commercially available bone cements**
Tiffany Lucas (Smith & Nephew GRC, York Science Park)

3.15 **Alterations in dendritic spine morphology in the hippocampus of aged rats 45 minutes after induction of long term potentiation.**
T.M.Dhanrajan (Brain Research Group, Open University)

3.30 Tea **ADJUDICATION**

4.00 **Limits of Technology; where are the frontiers ?**
Paul Monaghan (Royal Microscopical Society)

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Please reply to

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I hope to be present at the SEMT meeting on 11 June 1997

Name ..... Phone .....

Address .....

S.E.M.T. 11 June 1997 Beginners' Meeting

Microscopes & Examination of Jurassic Parks

Phil Wilby
British Geological Survey

Soft parts of animals are rarely preserved as fossils, but in Jurassic amber, from 35-40 million years ago, leaves and a gecko have been preserved. At Cambridge the amber has been split open; the tissues were still soft (though not well preserved structurally), and DNA was extracted.

Fossils of Archaeopteryx show impressions of the feathers; otherwise the skeleton is very similar to Composognathous in the same deposits.

Good preservation can be obtained if apatite replaces the tissue, as calcium phosphate, e.g. in microfossils. In deposits from N.E.Brazil from 100 million years ago - dinosaurs etc - even the gills and cells of the stomach wall may be well preserved; when the stone is split, a fish often jumps out entire. The roads may be made of fossiliferous rocks. Some artefacts are now made by the natives !

Wilby has done experiments on decay rates. After 2-3 days, muscle breaks up into sqare blocks, such as may be found in pterosaurs. The calcium apatite nucleates in the structure; some specimens fossilised during rigor mortis. In some other specimens, the bacteria around individual fibres have fossilised. Soft tissues may be only lightly phosphated; more phosphate may be found in the skin and outer layers, because phosphate has been absorbed from surrounding saturated water. At Bristol they have found that an animal in absolutely anoxic water with phosphate, will fossilise in the laboratory.

Pyrites (FeS) and calicite (CaCo3) are only present in soft tissues from two places, from Lavalt, France. Here the tissues were first phosphatised, then replaced by calicite and pyrite.

Amber fossils must usually be broken open; it is very difficult to dissolve the resin. The resin has some antiseptic properties.

Volcanic ash can help to preserve because it covers the animal so quickly.

Shrimps must be in completely anoxic surroundings in order to have the phosphate soluble. Well-preserved shrimps may be found inside fossil fish; if sellotape is applied and ripped off several times, internal organelles can be seen.

Microscopy of biofilms on intraocular lenses

Louise McLaughlin-Borlace
Inst. of Ophthalmology, Leeds

Lenses may now be inserted into the eye with cataract operations, but may get infected, and the bacteria then produce a biofilm over the lens. This may be stained with glutaraldehyde/ruthenium red. Of 27 lenses removed because of failure, 14 were infected; the infection is difficult to treat with antibiotics because of the biofilm protection.

Fully hydrated biofilms can be examined by Atomic Force Microscopy. The lenses may have bacteria on them for 12 years before inflammation and biofilm occur. Were the bacteria in the eye before the lens was put in ?

Future work will check which lenses are better from this point of view; some plastics may be better than others.

Characteristics of 2 commercially-available bone cements

Tiffany Lucas
Smith & Nephew GRC, York Science Park

Bone cement is a 2-part polymer to hold a prosthesis in place; it sets after mixing. It consists of polymer beads + opacifier, which together form a powder, with a zirconium salt or barium sulphate.. The polymer beads are smooth, spherical, 70 µm diam. The opacifiers differ in shape and elemental content; they have random distribution in the set cement.
The cement must be hard, long-lasting, ,and not brittle. In practice a prosthesis generally only lasts about 10 years anyway.

Alterations in dendritic spine morphology in the hippocampus of aged rats 45 minutes after induction of long-term potentiation

T.M. Dhanrajan
Brain Research Group, Open University

Long-term potentiation in the dentate gyrus region of the hippocampus was studied. Golgi stain was used - the Golgi does not stain in all fibres. Dendrite spine length was measured.

Limits of Technology; where are the frontiers ?

Paul Monaghan
Royal Microscopical Society

Morphological studies are powerful, but considered old-fashioned by grant-givers; functional applications and locations are needed.
Confocal microscopy is not an alternative to EM; it only demonstrates the presence of a particular fluorescent protein.
There is very little biological use for FE, High Resolution TEM; we need better resolution on elemental analysis.

X-ray microanalysis can be done on quite thick sections; EELS only on very thin sections.
Digital high-resolution camera can digitise the image, store it, and the output is publishable.
SEM + Field Emission is marvellous !
Environmental SEM can be used for uncoated, frozen etc specimens, and has a higher imaging capacity. Even live insects can be studied. Very useful at the Natural HIstory Museum. Specimens can be freeze-fractures and sublimed in the EM. There are limited applications in some fields.

The new Philips EM can do red/green 3-D images on screen.

For fucntion/location, we can use probes - antibodies, tags, endogenous labels.
The confocal image only says "probably is...."
Liu in Slot's lab has a different way of picking up frozen sections, which gives a less fuzzy image.
Apoptosis is usually easy to see with Toluidine blue staining.
High pressure freezing can be used for relatively large pieces of tissue, relatively easily; then substitute or freeze-dry.

Specimen preparation techniques are slill not completely sorted out.

Everyone's problem is money and manpower.
Technical developments must be commercially viable.

Atomic Force Microscopy will probably produce spectacular pictures.

SEMT Beginners' meeting

Abstracts
(copied as supplied)

Jurassic Parks under the microscope

Phil Wilby (British Geological Survey, Keyworth, Nottingham)

Normally, only the "hard parts" (bones and teeth, or shells) of ancient animals are preserved in the fossil record. However, in a few rocks ranging in age from over 500 million years to the present, the "soft parts" (e.g. muscle, gills, gut etc) of fossils are also preserved. The most spectacular preservation occurs where the soft tissues have been replaced by apatite (a calcium phosphate). In these cases, the entire soft tissue anatomy of organisms such as fish, dinosaurs, pterosaurs and certain crustaceans may be replicated. Microscopic examination of such material has provided valuable insights not only into the biology and ecology of these animals, but also the mechanism of fossilization. Using both scanning and transmission electron microscopy, it has been possible to demonstrate that such soft tissues are preserved at the sub-cellular level, and that phosphatisation was very substrate-specific, precipitating only on certain biomolecules in a process akin to pathological biomineralisation. EDX analysis of individual fossils demonstrate clear gradients in the degree of phosphatisation of the soft tissues from a high at the periphery of the fossil (i.e. within the skin), to a low at some greater depth within the carcass. This suggests that the phosphorus diffused into the carcass from an external source down its concentration gradient. Comparison of the fossil material with critical point dried tissues from controlled decay experiments suggest the infiltrating phosphorus to have fossilised the carcasses within 55 hours of death!

Microscopy of biofilms on intraocular lenses

Louise McLaughlin-Borlace (Inst. of Ophthalmology, University College, London)

PURPOSE
This project aimed to image bacterial biofilms on intraocular lenses (IOL's) removed from non-infected patients at Moorfields Eye Hospital (MEH)
We compared 3 microscopy techniques: Hoffman Modulation Contrast Light Microscopy, (LM), Atomic Force Microscopy (AFM), and scanning electron microscopy, (SEM).
METHODS
For LM and AFM, IOL,s were collected in sterile saline, while for SEM, IOL,s were fixed in 2.5% glutaraldehyde, O.5% ruthenium red, in cacodylate buffer, processed as normal for an SEM and viewed under a Jeol JSM 6100 SEM. Hoffman Modulation Contrast optics mounted on a Nikon Labophot microscope with long working distance water immersion lenses were used to image unfixed lenses at CAMR, Salisbury. For atomic force

microscopy, lenses were taken to Portsmouth, and imaged unfixed, using a TopoMetrix Discoverer AFM.

RESULTS

LM gave a good overview of the presence of biofilm on the IOL's at a low magnification (x20, x40). AFM provided a more detailed view of small areas of the biofilm, and its image analysis proved useful. SEM provided a high magnification view of the bacteria and dehydrated polymer matrix.

CONCLUSIONS

The techniques tested proved complementary. LM provided a quick method of screening IOL's for biofilm, whereas AFM proved to be an attractive non-invasive technique, which provided detailed information about the biofilm. SEM using ruthenium red fixative preserved the biofilm in a dehydrated form. The main advantages of AFM and LM over SEM, were that they enabled the study of biofilms in their natural, fully hydrated state.

Characterisation of two commercially available bone cements

Tiffany Lucas (Smith and Nephew GRC, York Science Park, York)

The aim of this work was to characterise two commercially available bone cements.

Bone cements are acrylic substances used in orthopaedic surgery to seat and fix the prostheses to the bone. Bone cements basically consist of two components, a co-polymer powder and a liquid monomer. The two components are mixed together to set off the exothermic polymerisation reaction. After the mixture is applied it sets to become a hard polymer.

Initially the powder components were then examined in a Jeol JSM6400 scanning electron microscope. Low accelerating voltages and large spot sizes were used because the co-polymer powder was easily damaged under the beam. X-ray microanalysis was carried out on the samples where spectra and maps were obtained. The components of the powder were examined separately. A separation technique was used using a density column. The polymer beads were measured using image analysis.

The set cements were examined also. Secondary and backscattered imaging were carried out. A higher accelerating voltage and smaller spot size was used to excite the heavier elements (the opacifier) in the cement. X-ray mapping was carried out on the samples to determine the distribution of the opacifier within the polymer matrix.

The results show that the main morphological differences between the set bone cements are in the opacifier distribution within the polymer matrix. There is little difference between the polymer beads used in the bone cements.

Alterations in dendritic spine morphology in the hippocampus of aged rats 45 minutes after induction of LTP

T.M.Dhanrajan (Dept of Biology, Open University, Milton Keynes)

Long term potentiation (LTP) was induced unilaterally in the dentate gyrus of aged (22 months) rats by tetanic stimulation of the perforant path (3 trains of 250 Hz for 200 millisec). Test shocks (1/30 sec were delivered for 10 min before, and 45 min after tetanic stimulation. The mean increase in population EPSP slope in the last 10 min of the experiment compared to the 10 min prior to tetanization was 119% +2.89 SEM). At the end of the recording period animals were perfused with 2% glutaraldehyde and 2% paraformaldehyde, and Golgi-stained preparations made from 1 mm slabs cut transversally to the septo-hippocampal axis. The area selected for analyses was the medial molecular layer of the dentate which contained dendrites of granule cells. 15-20 granule neurons from each hemisphere of the five rats were analysed using computerised image analysis and tilting dissector method (Rusakoff, & Stewart, J. Neurosci. Method. 60, 11-21). Identification and quantification of spines was performed using a segmentation alogrithm and a line skeletal transformation of dendritic images.

Analysis of the data indicates a significant (p 0.026) decline in spine length (10%) in the potentiated compared to the unpotentiated hemisphere. However, whilst the mean spine density along dendrites was approximately 6% lower in the potentiated hemisphere, this difference was not significant. The distribution of the inter-spine distances showed;
(a) The presence of dense spine groups ("collars") and
(b) no inter-hemispheric differences.

These preliminary data indicate that in older rats dendritic spine plasticity after LTP has similarities to that shown in our previous studies with younger rats.

Limits of technology: where are the frontiers ?

Paul Monaghan (Royal Microscopical Society)