SEMT

Society of Electron Microscope Technology

ONE DAY MEETING

Wednesday 13th December 2006

at the

School of Pharmacy

University of London

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2007 Programme: 4th April (Half Day Meeting) **& 12th December** (One Day meeting and AGM)

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The Programme

09.15 a.m.
Registration, Tea/Coffee, Trade Exhibition

10.00 a.m.
Introduction: Chair, Heather Davies.

10.05 a.m.
"Visualising Pharmaceutical Nanosystems".
Professor Ijeoma F. Uchegbu, Pharmaceutics Dept. School of Pharmacy.

10.45 a.m.
"Looking up from the basement: The role of EM in the diagnosis and investigation of inherited skin diseases". Trish Dopping-Hepenstal, St John Inst. of Dermatology, St. Thomas' Hospital.

11.35 a.m.
Tea, Coffee.

11.55 a.m.
"Dual beam analyses of Stardust" (cometary material).
Dr. John Bridges, Planetary and Space Science Research Inst., Open University

12.35 p.m.
Buffet Lunch, Trade Exhibition

2.00 p.m.
"Structure and function of transcription and replication factories in human cells".
Dr. Dean Jackson, Faculty of Life Science, University of Manchester.

2.40 p.m.
"A New Angle on Diamond Knives"
Helmut Gnägi, Diatome, Switzerland.

3.30 p.m.
"Modelling a tissue: polarised light and scanning electron microscopy of articular cartilage".
Louise Hughes, Aberystwyth, University of Wales.

4.10 p.m.
Tea, Coffee.

4.40 p.m.
"A Nosoconial outbreak of Pox Virus in Asia".
Barry Dowsett, Health Protections Agency, Porton Down, Salisbury.

5.00 p.m.
Annual General Meeting

5.30 p.m.
Wine Reception, followed by the Conference Dinner.

Visualising Pharmaceutical Nanosystems

Ijeoma F. Uchegbu, Department of Pharmaceutics, School of Pharmacy, University of London, London WC1N 1AX

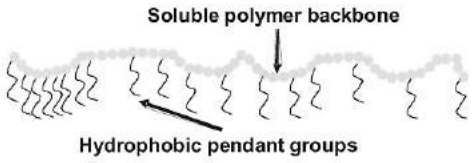

Fig. 1: Schematic representation of comb type amphiphilic polymers

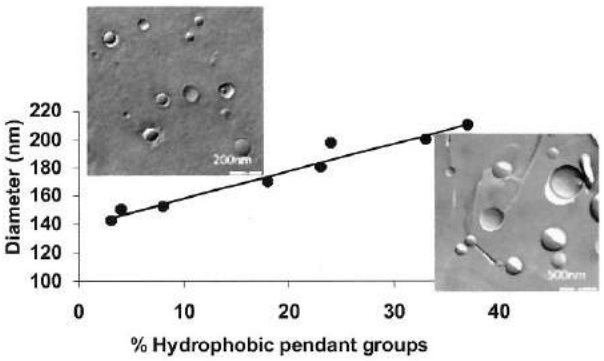

Fig. 2: Comb type amphiphilic polymers self assemble into vesicles, increasing the number of hydrophobic pendant groups increases vesicle size.

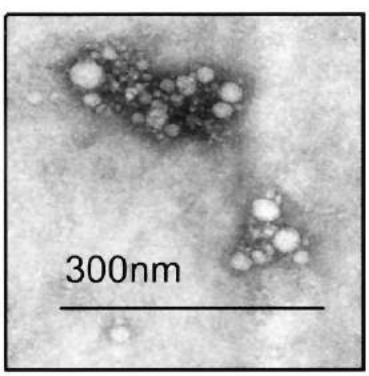

Fig. 3: Comb type amphiphilic polymers self assemble into micellar clusters and increase the brain activity of drugs by ten fold.

Pharmaceutical nanoscience is concerned with improving the activity of medicines by exploiting nanoscience concepts. Packaging drugs and genes into nanosystems enables drug or gene biodistribution to be favourably altered with an ultimate therapeutic benefit (1-3). A ten fold increase in drug activity may be obtained (2) and an effective anti-cancer gene medicine is achievable (3). To acquire such control on the in vivo fate of drugs and genes requires that such particles be precision engineered and electron microscopy allows researchers to visualise and confirm the results of such engineering. Pharmaceutical nanosystems result from molecular self assembly and in our laboratories we have focused on controlling nanosystem parameters by exercising control on the chemistry of the self assembling amphiphiles which are comb type polymers (Figure 1).

We have been able to identify two chemical features of these amphiphilic polymers which control nanosystem size (and ultimately nanosystem biodistribution) and these are the molecular weight of the polymer (4) and the level of hydrophobic substitution of the polymer (5), both of which are positively and linearly correlated with nanosystem size (e.g. Figure 2). Additionally the level of hydrophobic substitution on a water soluble polymer ultimately dictates the nature of the resulting self assembly and as such morphologically distinct nanosystems such as dense nanoparticles, polymeric vesicles and micellar aggregates are possible from one polymer backbone (5, 6), with each type of self assembly having a different drug delivery profile. For example micellar clusters (Figure 3), in which multiple micelles are linked to one another, improve the brain activity of drugs by up to 10 fold (2).

This research is focused on correlating polymer chemistry with the nature of the resulting self assembly and ultimately linking a range of morphologically distinct self assemblies with specific drug delivery function.

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2. Qu X.; Khutoryanskiy V. V.; Stewart A.; Rahman S.; Papahadjopoulos-Sternberg B.; Dufes C.; McCarthy D.; Wilson C. G.; Lyons R.; Carter K. C.; Schatzlein A. G.; Uchegbu I. F., Biomacromolecules in press, DOI: 10.102/bm0604000.
3. Dufes C.; Keith N.; Bisland A.; Proutski I.; Uchegbu I. F.; Schatzlein A. G., Cancer Res. 2005, 65, 8079-8084.
4. Wang W.; McConaghy A. M.; Tetley L.; Uchegbu I. F., Langmuir 2001, 17, 631-636.
5. Wang W.; Qu X.; Gray A. I.; Tetley L.; Uchegbu I. F., Macromolecules 2004, 37, 9114-9122.
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Looking up from the basement: The role of EM in the diagnosis and investigation of inherited skin diseases.

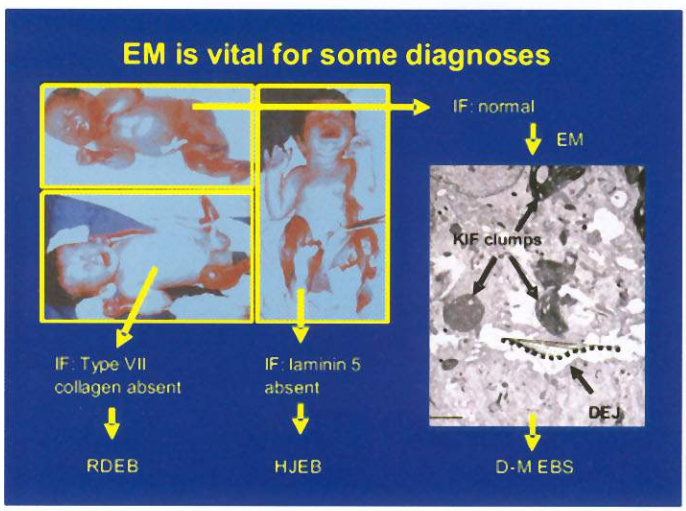
Trish Dopping-Hepenstal,
National Diagnostic Epidermolysis Bullosa Laboratory, Guy's and St Thomas' NHS Foundation Trust.

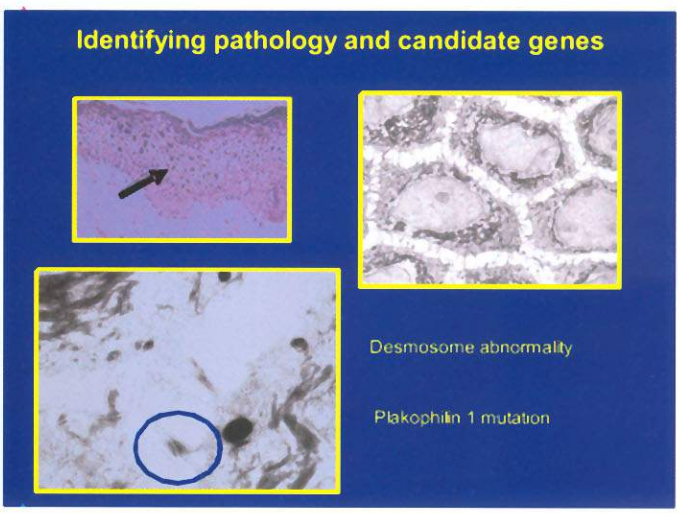
Despite the development of techniques for immunohistochemistry and molecular genetics, electron microscopy still plays an important part in the diagnosis and study of hereditary skin disorders, such as epidermolysis bullosa (EB). In EB, the upper layers of the skin do not adhere normally to the lower layers, resulting in fragile, blistering skin. There are three major types of EB and a number of subtypes; most of the abnormalities occur in the basement membrane zone of the dermal-epidermal junction.

Diagnosis of some conditions relies on ultrastructural examination and cannot be made by other means. In some cases, this is because the antibodies for diagnosis by immunohistochemistry are not very reliable or not widely available (e.g. anti-plectin for junctional EB with muscular dystrophy and anti-kindlin for Kindler syndrome). In other cases, there is no suitable immunological marker and diagnosis relies on the identification of characteristic ultrastructural features (e.g. the Dowling Meara form of EB simplex – identified by the presence of characteristic keratin intermediate filament clumps). Immunohistochemistry is not helpful in conditions where a mutation causes subtle changes in the structure of a protein but not the quantity produced (e.g. striate palmo-planter keratoderma, where a missense mutation causes keratin filament condensation in suprabasal cells). Hence, EM is essential for routine diagnosis, both for patient care and for the identification of candidate genes for mutation analysis, as well as for prenatal diagnosis by foetal skin biopsy.

EM also plays an essential role in studies to improve patient treatment, such as clinical trials of treatments to improve healing of lesions in recessive dystrophic EB.

EM has lead to the recognition of pathology. The first desmosomal abnormality identified was in plakophilin and the clue to the nature of the pathology came from EM. There are still proteins out there yet to be characterised, and there are proteins that have been characterised but the pathology has not yet been recognised, so EM will continue to be important in the future.





New Techniques for MicroAnalysis of Cometary and Asteroidal Material Using Dual Ion and Electron Beam Microscopes

John Bridges
Planetary and Space Sciences Research Institute, Open University, Milton Keynes MK7 6AA.

In order to fully characterize returned cometary and asteroidal samples they need to be studied down to sub-micron scales in order to fully understand their formation and what processes of any alteration they have undergone. As an example of this analytical problem within meteorites, fine-mineral intergrowths which constrain the heating conditions and reactions that the sample has undergone can only be fully resolved at sub-micron levels (Bridges et al. 2004).

Dual Focused Ion Beam/Scanning Electron Microscope (FIB-SEM) analyses now allow the characterization and physical separation of sub-micron sized particles (Fig. 1). This is, for instance, being used in the Stardust mission studies at the Open University to extract cometary residue from impact microcraters (e.g. Bridges et al. 2006). This material can in turn be used with transmission electron microscopy for nanometre-scale resolution of mineralogical features and X-ray analysis. Alternatively, extracted samples can be used for mass spectrometers (e.g. C, O isotopes) or other geochemical analyses. This work will continue to be developed and will form a crucial part of asteroidal and Mars sample return studies. It will also help detailed comparisons to be made with known meteorite groups to help ascertain how representative our meteorite samples are of asteroids.

Advances in TEM technology are also improving sample image resolution and new equipment obtained with SHRIF funding at the Open University and other UK academic institutions will allow compositional analyses and textural information to be obtained from material prepared with FIB-SEM systems. One of the advantages over traditional methods of thin wafer preparation for TEM analyses is the ability to characterize and select micron-sized areas for extraction and further study. The fine-grained nature of the likely mineral assemblages that will be encountered in the NEAR mission mean that FIB-SEM based analytical procedures will be an essential tool.

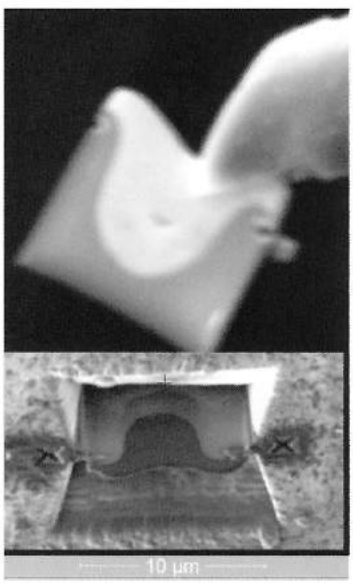


Fig.1. A 10 um wide, 1 um thick wafer being produced from a hypervelocity microcrater for detailed EDS X-ray, TEM or isotopic analyses. Below shows outline of crater revealed after ion beam thinning for subsequent EDS analyses of the residue. Above shows micromanipulator holding the extracted section of the crater. Instrument FEI Quanta 200 3d Dual FIB-SEM with Xtreme Access **manipulator for sample extraction.**

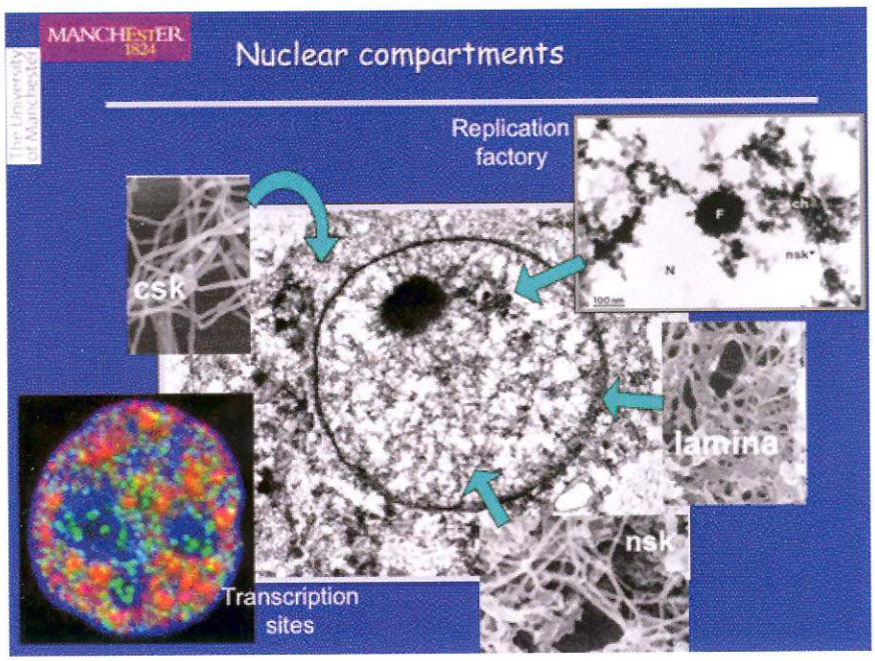
Bridges J.C, Franchi I.A. and Green S.F. 2006 Extraction and analysis of microcrater residues by focused ion beam microscopy. LPSC, #1664.

Bridges J. C., Warren P. H. and Lee M. R. 2004. Olivine Decomposition Features in the Y000593 and NWA998 Nakhlites. Meteoritics and Planetary Science 39, #5140.

Structure and function of transcription and replication factories in human cells.

Dr. Dean Jackson, Faculty of Life Science, University of Manchester.

It is generally accepted that the nuclei of higher eukaryotes are structured so that fundamental functions such as RNA synthesis and processing and DNA replication and repair are performed within specialised nuclear compartments. The importance of these compartments could be of fundamental importance in regulating different aspects of nuclear function. However, the molecular principles that define this potential level of regulation are still a matter of intense debate. My talk will look into this intriguing question by focusing on the interplay between the key nuclear compartments where DNA and RNA synthesis are performed and the nucleoskeleton, which is a major candidate for organising the architecture of these sites. I will describe how different electron and light microscopy techniques must be used in conjunction with molecular cell biology approaches in order to achieve clear insights into the complex behaviour of nuclei in mammalian cells.



A New Angle on Diamond Knives

Helmut Gnägi, Diatome, Switzerland.

Ultramicrotomy has been a routine procedure for TEM for over 50 years. During this time there have been improvements to fixation and embedding procedures but little advances in knife technology since Latta and Hartman introduced glass knives in 1950 and Fernadez Moran produced the first diamond knives in 1954. In this talk the influence of knife angle on structure preservation within tissues will be discussed for room temperature and cryo applications and old concepts and boundaries will be challenged. Examples of advantages for both biological and materials sciences samples will be presented and concepts such as the benefits from oscillating the diamond during cutting and electrostatic charge reduction will be considered. Finally there will be examination of how far you can go in reducing knife angle to reduce compression but retaining knife integrity and life expectancy between resharpening.

A Nosoconial outbreak of Pox Virus in Asia.

Barry Dowsett, Health Protections Agency, Porton Down, Salisbury.

In 2006, a multi-centre outbreak of a poxvirus-like infection occurred in burns patients in Karachi. Atypical lesions involving a burns wound were first noted in a patient at one of the burns units within the City. Similar lesions rapidly affected other patients within this unit. Subsequent reports of similar infections were received from other burns centres in Karachi. Patient samples were sent to the Centre for Emergency Preparedness and Response, Porton Down for investigation. This presentation will describe the application of electron microscopy and other techniques in the definitive diagnosis of this disease outbreak.

Modelling a tissue: polarised light and scanning electron microscopy of articular cartilage.

Louise Hughes, Aberystwyth, University of Wales.

Studies on the structure of articular cartilage have been ongoing since William Hunter's (1743) original investigation describing the tissue as "velvet-like". Many microscopical techniques have been used to study the tissue under a variety of conditions, but these investigations frequently contradict one another and few have attempted to relate the structural appearance of the tissue to its function. This is due mainly to the fact that the ultrastructural preservation of articular cartilage presents a substantial challenge. The tissue is avascular, aneural, has an extensive extracellular matrix that maintains a constant internal pressure of approximately 2 atmospheres (Urban et al., 1979; Basser et al., 1998) and a very low oxygen concentration (Sliver, 1975), which inhibits the action of some of the traditional chemical fixatives. Using both optimised chemical fixation and cryo-preservation methods in combination with different imaging techniques, a model of cartilage was constructed (Hughes et al., 2005) that incorporated information from the macroscopic level of the joint through to the ultrastructure of the extracellular matrix that provides the biomechanical properties essential to the tissue's functionality.

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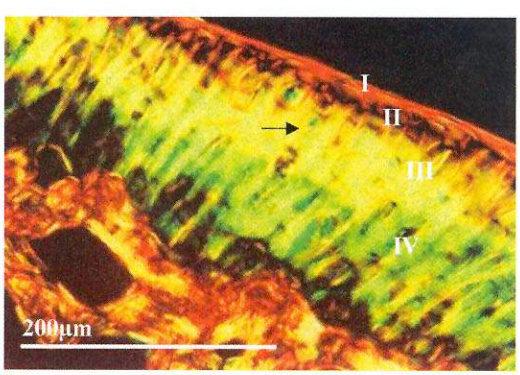

Figure 1 shows adult mouse articular cartilage, fixed in formaldehyde in PIPES buffer (pH 8.5), dehydrated in ethanol, embedded in wax, sectioned, stained with picrosirius red and imaged by polarised light microscopy. Each of the classical histological zones are present, the superficial (I), intermediate (II) deep (III) and calcified zones (IV) and columns of chondrocytes, lacking birefringence and surrounded by bands of territorial matrix (arrow), extend through the deep zone and calcified zone.

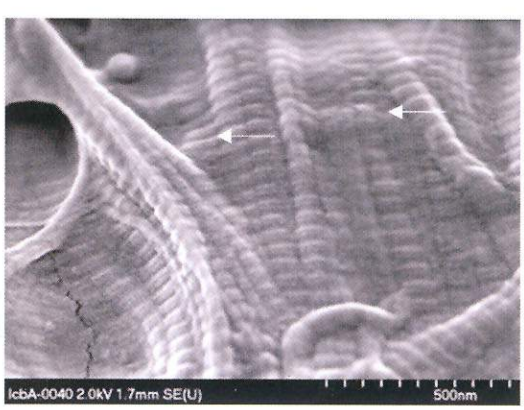

Figure 2 shows the territorial region (surrounding chondrocytes) of the collagen extracellular matrix in the deep zone of cryofixed adult mouse articular cartilage, freeze substituted, dried and coated and imaged with a field emission scanning electron microscope. The collagen fibrils are predominantly parallel to each other and positioned so that the d-periodic banding appears to be aligned. Orthogonally arranged fine fibrils are also aligned on the d-periodic banding and can be seen to span between some of the main collagen fibrils (arrow).