



MICROSCOPY
OF
BIOMATERIALS II

ABSTRACTS

Scientific Organisers:
Dr S. Best (Queen Mary & Westfield College)
Dr J. Czernuszka (University of Oxford)
Dr I. Turner (University of Bath)

PROGRAMME

1000-1020	Registration and Coffee	
1020-1030	Welcome and Introduction	
1030-1115	S. Mann	Biominerals and biomimetic materials
1115-1130	P. Wyeth	Durable bioceramics: the teeth of the Australian parrotfish
1130-1145	F. Vollrath	Structural hierarchy of spider silk
1145-1230	L. Hench	Microscopy of bioactive glasses: a review
1230-1245	Discussion	
1245-1400	Lunch / POSTERS / Exhibition	
1400-1445	M. Freeman	Wear debris and osteolysis
1445-1500	P. de Aza	Electron microscopy study of a wollastonite-tricalcium phosphate Bioeutectic® material
1500-1515	J. Huang	Evaluation of <i>in vitro</i> performance of Bioglass®/polyethylene composite by microscopy
1515-1530	C. Scotchford	Application of confocal microscopy to the study of bone cell responses to biomaterial surfaces
1530-1545	H. Gledhill	<i>In vitro</i> fatigue testing of vacuum plasma sprayed hydroxyapatite coated implants aged in Ringer's solution
1545-1600	D. Gordon	Evaluation of scanning electron microscopy for use in the study of the bone-biomaterial interface
1600-1615	A. Minnoci	Permeability versus porosity in microporous small-diameter vascular grafts made by a 'spraying, phase inversion technology'
1615-1630	I. Schmitz	Ultrastructural investigation of vascular grafts
1630-1645	Discussion	
1645-1700	Tea and POSTER PRIZE	
1700	CLOSE OF MEETING	

POSTER PRESENTATIONS

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| P1 | D. Corrand | The effect of some C ₃ -saturated organic molecules on the <i>in vitro</i> precipitation of hydroxyapatite |
| P2 | H. Gledhill | Morphological comparison of hydroxyapatite coatings produced by two different thermal spray techniques |
| P3 | P. Hatton | Ultrastructure of glass-ionomer (polyalkenoate) cements in the transmission electron microscope |
| P4 | C. Hodges | Thermal microscopy of biofilms |
| P5 | A. Lawson | Diffusion controlled precipitation of calcium phosphate on collagen |
| P6 | A. Minnoci | Microscopical evaluation of nerve guidance channel internal surface microgeometry and material biostability |
| P7 | J. Minns | Microscopical investigation of metal-on-metal wear of removed polyethylene-on-metal total knee prostheses |
| P8 | P. Mummery | Failure mechanisms in natural composites |
| P9 | B. Shahgaldi | Tissue metallosis caused by corrosion of stainless steel fracture fixation devices |
| P10 | F. Vollrath | Structural elements of spider silk investigated with transmission electron microscopy |

Specimens were embedded in LR white resin, polished to reveal implanted material and carbon coated for examination. SEM examination was carried out using the backscatter and secondary electron imaging modes and the results compared to those obtained using light microscopy. Scanning electron and light microscopical techniques used clearly indicate differences in bonding behaviour between these cements and provided a method for observing the undisturbed bone-biomaterial interface. The method offers the additional possibility of applying elemental analysis to establish possible ion movements in and around the interface. It was concluded that SEM of polished resin embedded specimens provided a suitable method for evaluating the bone-biomaterial interface.

Permeability versus porosity in microporous small-diameter vascular graft made by a 'spraying, phase-inversion technology'

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Porosity is a key feature in synthetic small-diameter vascular graft (SDVGs) design and development. To manufacture porous SDVGs we used a novel technology called spraying, phase-inversion (SPI) which raises the question of how to evaluate the porosity of these grafts. Stiff grafts made of Dacron and PTFE are traditionally evaluated by water permeability (WP). However, grafts made by SPI feature a filamentous, sponge-like structure and therefore the relationship between WP and porosity is not known. To investigate this issue WP was evaluated by measuring the volume of de-gassed water filtering through the graft wall, at 120mmHg of pressure, in one minute. SEM digitized slow-scan imaging was used to estimate the percentage of luminal and external surface open area. By an image analysis system a grey value threshold was applied to discriminate open from closed area and to calculate their surface ratio. Preliminary data indicate a direct correlation between WP and percentage of open area.

Ultrastructural investigations of vascular grafts

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Scanning electron microscopy is a useful tool to investigate the incorporation pattern of synthetic vascular grafts. The aim of our study was to demonstrate the structure of native prostheses and observe the pattern of incorporation of vascular grafts. Next to native grafts we investigate 32 Dacron and Teflon grafts with incorporation intervals between 9 days and 22 years (Dacron bundle length approx. 0.4mm; width approx. 0.3mm; Teflon fibrils length 2.6-22.6µm). We found: i) early phase with suffusion of graft material by plasma and fibrin; ii) phase of organisation with beginning neovascularisation; iii) late phase with scar-tissue formation. Through all these phases there is evidence of a foreign body reaction against the graft leading to destruction of the synthetic material in some cases. An inner (neo-intima) and outer (fibrous) coating of the graft material could be identified.

thought to develop as a result of polyacid degradation during the setting reaction. This evidence supported current theories on the setting chemistry of GICs and could be used to develop a model for their biocompatibility.

Thermal microscopy of biofilms

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The new technique of scanning thermal microscopy (SThM) involves passing a small heated tip over the sample and monitoring the heat that is lost using a feedback loop. This then gives information as to the distribution of the thermal conductivity across the sample near the surface. Two types of sulphate reducing bacteria, which have been isolated from steel pipelines, have been grown on steel substrates. These samples were then observed by SThM. Preliminary results show that the variation in contrast in the image is due to changes in the thermal conductivity between the bacteria and the substrate. Contrast was also seen in the bacteria. The aim is to use the bacteria as a means of protection for steel pipes etc. to reduce the rapid corrosion that takes place when strong acids are passed through the pipes.

Diffusion controlled precipitation of calcium phosphate on collagen

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A synthetic bone substitute comprising calcium phosphate and collagen has been produced by a method analogous to the formation of natural bone. Calcium phosphate is precipitated onto collagen sheets by the diffusion of calcium and phosphate ions through the collagen membrane. Characterisation of the composite has been carried out using electron microscopy, X-ray diffraction and infra-red spectroscopy.

Through variation in the pH of the precipitation solutions, complete coatings of both octacalcium phosphate and hydroxyapatite have been produced. The diffusion rates of the two ions through the membrane are not equal and the coating forms primarily on the phosphate side of the membrane. Adjustment of the relative concentrations of the two ions has been used to control the diffusion rates and hence select the site of calcium phosphate precipitation.

Microscopical evaluation of nerve guidance channel (NGC) internal surface microgeometry and material biostability

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The peripheral nervous system (PNS) has regenerative capability. After an injury axons can repair the gap between the stumps if it is not too large. To avoid this problem surgeons use the technique of the suture under tension or nerve grafting supported by NGC. The influence of inner surface (IS) microgeometry of NGC on nerve regeneration has already been reported in literature. NOCs featuring a smooth IS allow a better regeneration of the nerve cable. We manufactured polyurethane (PU) NGCs with a highly smooth IS and evaluated them by AFM and SEM. AFM of PU-NGCs show roughness less than 600nm, whereas comparative silicon NGCs show roughness over 1400nm. Implantation experiments of PU-NGCs show, after 6-8 weeks, a good channel biostability and excellent regeneration of the nerve cable. SEM of the PU-NGCs show no evidence of microcracking in the external surface with a well regenerated nerve cable inside.

Microscopical investigations of metal-on-metal wear of removed polyethylene-on-metal total knee prostheses

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Eight cast cobalt-chrome total knee prostheses that normally articulate by a metallic femoral component on a concave polyethylene tibial component had dislocated *in vivo* because of gross instability, wear and breakage of the polyethylene. The two components then articulated by direct metal-to-metal contact as a consequence and were removed at revision surgery. The gross wear features and deformations were noted and the two metallic components cut for examination under light and scanning electron microscopy. The worn areas were first examined under differential interference contrast (DIC) conditions (as described by Nomarski) in the light microscope to show the surface morphology which is accentuated by this method. Adjacent to the primary wear area was a less worn darkened zone of 1-2mm width where contact was probably occurring intermittently; this zone had regular directional pits of