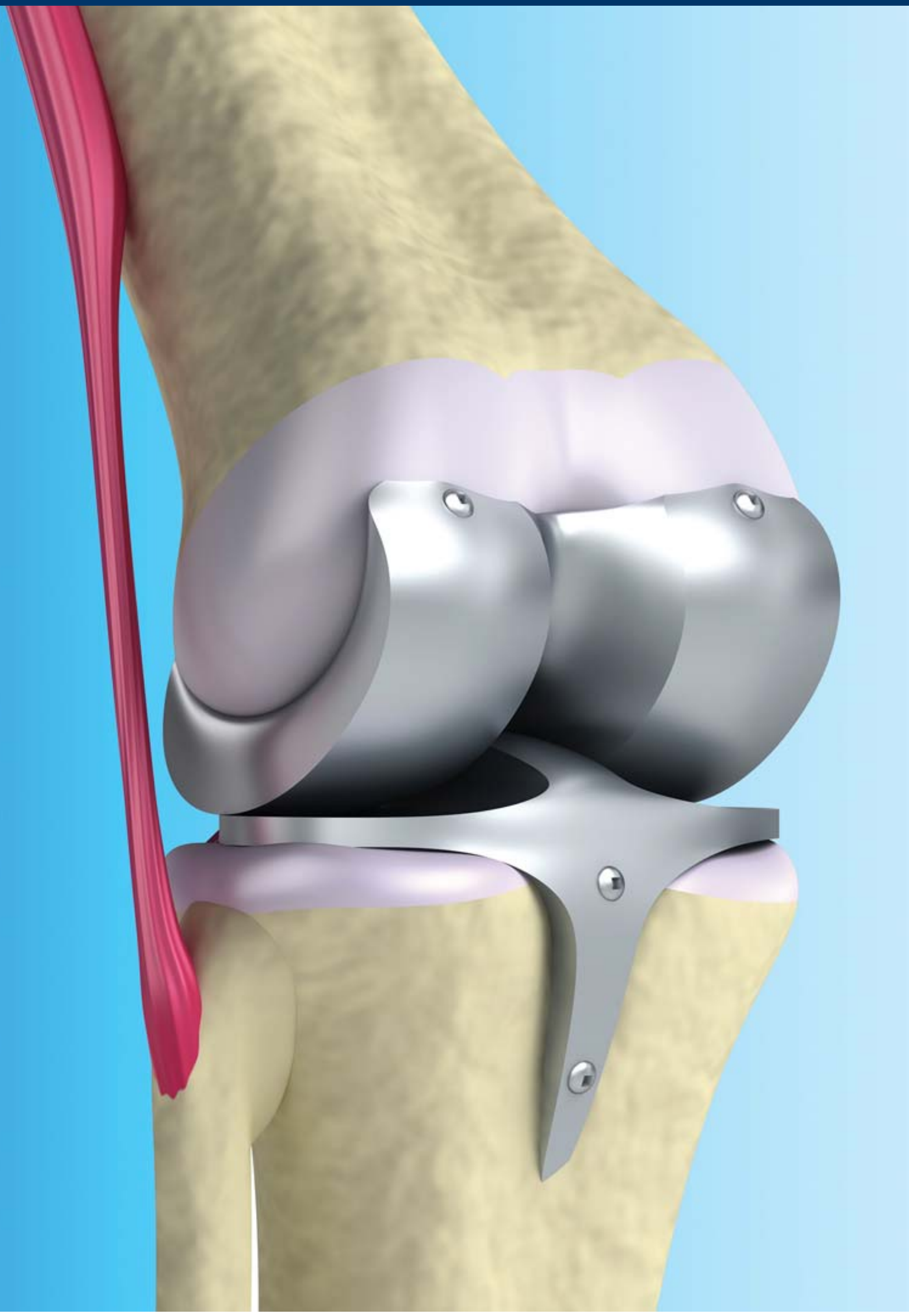
University of Glasgow

ISAAC
Imaging Spectroscopy And Analysis Centre

Cell Instructive Materials Improve Medical Implants

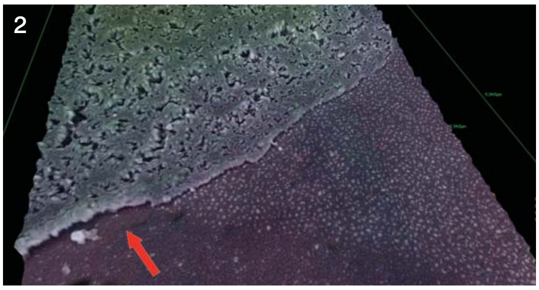


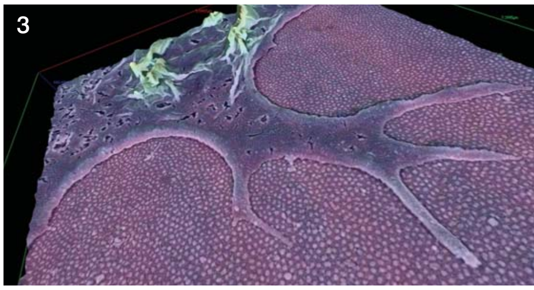
Artificial knee replacement

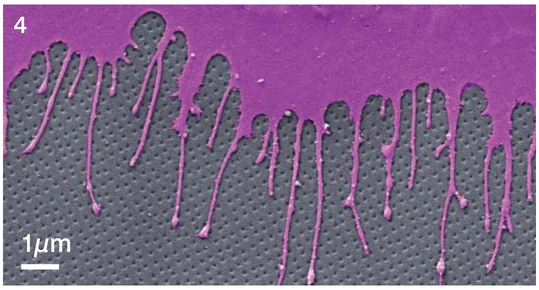
Assessing Cells Exposure to Composite Surfaces

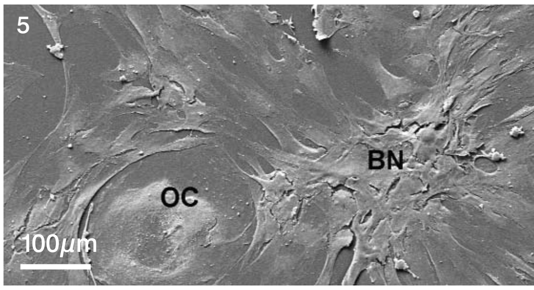
Materials scientists are designing novel surfaces that allow them control over cells. Composite materials where one part engenders bioactivity and the other part confers desirable characteristics (i.e., hardness, strength, etc.) are quite popular. Hydroxyapatite (HA) composite ($Ca_{10}(PO_4)_6(OH)_2$), which is analogous to bone mineral, is an example. Up to 50% of bone weight is comprised of bone mineral. Thus, HA is commonly used as a filler to replace amputated bone or as a coating to promote bone ingrowths into orthopaedic prosthetic implants.

Bone forming cells such as osteoblasts or their progenitors, mesenchymal stem cells, will produce bone apposed to HA. HA is very brittle (being a ceramic), so it is combined with polymers to provide a ductile fracture mode. As the composite is created, however, a polymer skin can form across the HA due to creep at high processing temperature, which is a problem. Techniques such as plasma cleaning or polishing are used to remove the skin and better expose HA to the cells. Low voltage field emission scanning electron microscopy (SEM) is ideal for determining what, and how much, of the HA is exposed to the cells and whether there is a strong cell response.









Understanding Cell Sensing Capability Limits

The University of Glasgow's Centre for Cell Engineering (CCE), in the Institute of Molecular, Cell and Systems Biology, working with the School of Engineering and other collaborators, is at the forefront of research in developing novel nano structured surfaces for stimulating the growth of mesenchymal stem cells. This will have a major impact in improving the manufacture of medical devices. As nano fabrication techniques become more advanced, the size of features can be significantly reduced. Nano features are rapidly gaining wider usage in biomaterials and tissue engineering, yet an understanding of cell response, while essential, is still nascent. Examination by SEM can be revealing. Supporting CCE's research is the Imaging Spectroscopy and Analysis Centre (ISAAC), in the School of Geographical and Earth Sciences (GES). ISAAC has two high resolution field-emission SEM's that can both operate in low vacuum. For flexibility with accurate analysis, one SEM offers an 'environmental' mode and the other SEM provides 3D images.

In orthopaedic materials, an area of focus for CCE, titanium (Ti) is widely used for load-bearing implants (i.e., hip and knee replacements). Its oxide layer (titania) makes it highly inert, however, and there is little bone growth on the implants, which results in micro motion (the cavity fills with soft tissue – bone marrow mesenchymal stem cells' default reaction) and eventual failure leading to the need for revision surgery in elderly or frail patients. By anodising them into the titania, nano features, which will induce bone formation from the mesenchymal stem cells, can be made with good levels of control.

ISAAC's SEM was employed to observe differences in cell response to nano feature heights of 8 nm and 15 nm. At 15 nm, the cells used large filopodia (long, slender cell cytoplasmic projections, or 'micro spikes') to induce high levels of bone formation. At 8 nm, however, the cells switched to using 'nanopodia' (short, thin cell projections) but bone formation reduced. This indicated the cells were at the very limits of their sensing capability and were 'hanging on by their fingertips' to use the spatial information available.

SEM Analysis of Complex Cell Cultures

In vivo, the situation is often more complex than just one cell type interacting with an implant material; thus, there is a move towards more complex cultures. Bone homeostasis is an example, where both osteoblast (bone forming) and osteoclast (bone removing) cells need to be considered as part of the natural tissue homeostasis. An additional complication is that osteoclasts are derived from fusing macrophages. When designing new orthopaedic materials, therefore, it is important to consider the homeostatic balance. The challenge then is to improve bone formation while osteoclastogenesis is not increased or reduced. SEM analysis has revealed co-cultures on nano scale surfaces demonstrating both bone nodule and osteoclast formation right next to each other.

CCE and ISAAC Expertise Provides Solutions for Cellular Response Characterisation

ISAAC's state-of-the-art facilities, combined with the expertise and knowledge of CCE, enable the University of Glasgow to offer comprehensive characterisation, analysis, and interpretation of cellular response at a micro and nano scale. In addition to analytical SEM and environmental SEM (ESEM), X-ray analysis and Raman spectroscopy are also offered. ESEM allows observation of cell response to fully hydrated gels, and X-ray and Raman analyses enable characterisation of, for example, bone mineral formation from deposition of HA layers. Rapid turnaround of analysis can also be provided to satisfy urgent client requirements.

ISAAC Contacts

School of Geographical & Earth Sciences
Gregory Building, Lilybank Gardens,
University of Glasgow, Glasgow G12 8QQ, UK

Peter Chung, Microanalyst
email: peter.chung@glasgow.ac.uk
Tel: 0141 330 5466 office / 5055 facility

www.glasgow.ac.uk/isaac

Images
1. *Artificial hip replacement*
2. *8 nm high islands in Ti – nanopodial projections interacting with the features, and in an area with no patterning, the cell is further from the material surface (arrow)*
3. *15 nm high islands in Ti – the cell is using filopodial projections to interact with the larger nano features*
4. *Osteoclast filopodia interacting with 100 nm diameter pits*
5. *Osteoclast / osteoblast co-culture with a large osteoclast (OC) next to a bone nodule (BN) made from mesenchymal stem cells, which are triggered to differentiate into osteoblasts by the underlying nano pattern*