Laser ablation machined sections permit correlative studies of HDMP by X-ray microtomography, optical and scanning electron microscopy

A Boyde, D Mills, LR Ranganath, JA Gallagher

Queen Mary University of London & University of Liverpool, UK

Problem

• Cracks in bone and articular calcified cartilage (ACC) of the subchondral plate heal with a High Density Mineralised Infill (HDMI) - which disappears with demineralisation, and thus becomes lost to knowledge, science, memory and even OARSI.
• HDMI also extrudes into hyaline articular cartilage (HAC) as High Density Mineralised Protrusions (HDMPs), which fragment and act as a cutting and grinding agent, damaging HAC from within.
• We wish to know more about the structure and composition of HDMPs.
• We recently prepared thin sections from the front face of bone blocks embedded in PMMA - previously studied by backscattered electron scanning electron microscopy (BSE-SEM) and X-ray microtomography (XMT) - by laser-ablation microtomy (LAM) and wanted to know if this method could be applied to this rather intractable problem.

Methods

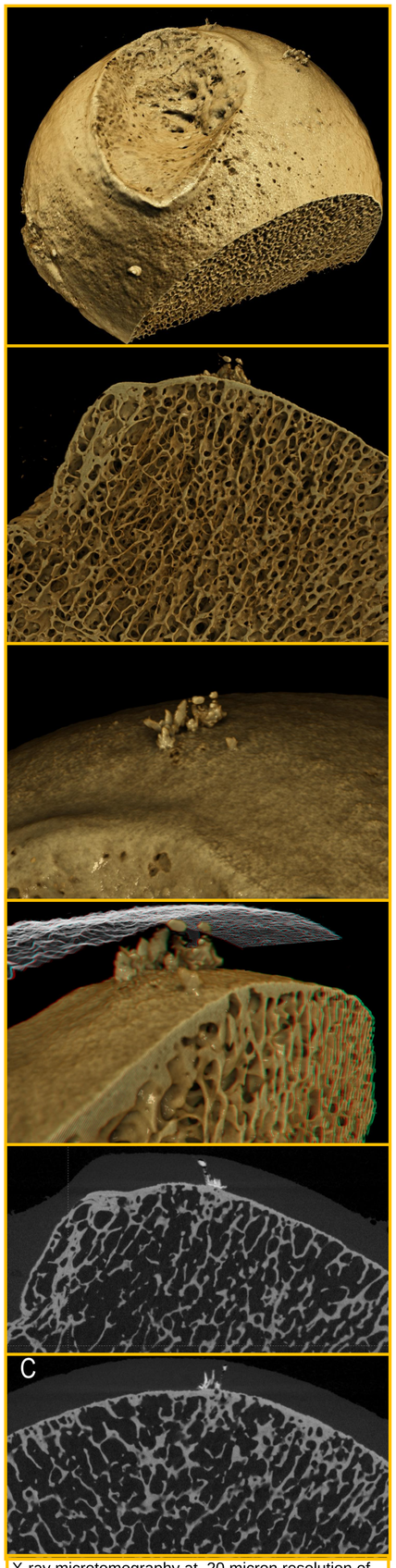
• Samples studied by DESS MRI and XMT before cutting slabs for PMMA embedding
• PMMA blocks reduced to include regions having HDMPs
• Polishing for BSE SEM and higher resolution XMT.
• Block surface in XMT reconstruction corresponds exactly with that seen by BSE SEM and what will be seen in the **LAM LM section**.
• block face stuck to glass slide using cyano-acrylate adhesive and placed on 'Tissue Surgeon' equipment (LLS Rowiak LaserLabSolutions GmbH, Germany).
• femtosecond pulsed 1030 nm laser scanned through a cutting plane in the specimen.
• Laser energy is focussed by a high numerical aperture objective lens into a very small specimen volume and for an extremely short period, generating a high instantaneous laser flux that obliterates the specimen only at the focal spot.
• focussed, pulsed laser beam is scanned along a 1mm line, and this scanned to cover the entire area of the block
• block thereby released from the slide
• leaving section stuck to the slide.
• gentle wet polishing on 4000 grit silicon carbide polishing paper removes 1-2 µm of cutting relief from section
• block face and the underlying few microns comprise the section
• blocks can be serially resectioned
• uncoated LAM section on slide is placed in the SEM for BSE imaging
• For LM, apply coverslip using glycerol, easily removed with water
• glycerol does not attack PMMA or cyanoacrylate
• do not use ethanol which may harm PMMA
• we can go back and forth from SEM to LM methods, including
• Polarised Light Microscopy (PLM)
• Many LM stains work with embedding resin left in place.
• XMT, SEM and LM images are married using homemade software.

Results

• Thinner and intact sections, at 6 to 10µm, cf. 50-100µm range in classical ground sections.
• Sections undeformed, intact, undecalcified - from PMMA block surfaces - so we have perfect correlation with
• x-ray microtomography (µCT) and
• any confocal LM of the block before cutting, and
• BSE-SEM for mineral content studies, and
• iodine stained BSE-SEM for soft tissue histology (cells, osteoid, cartilage, ligament, tendon, periosteum etc) and
• all LM methods, including staining, phase, polarised light etc.

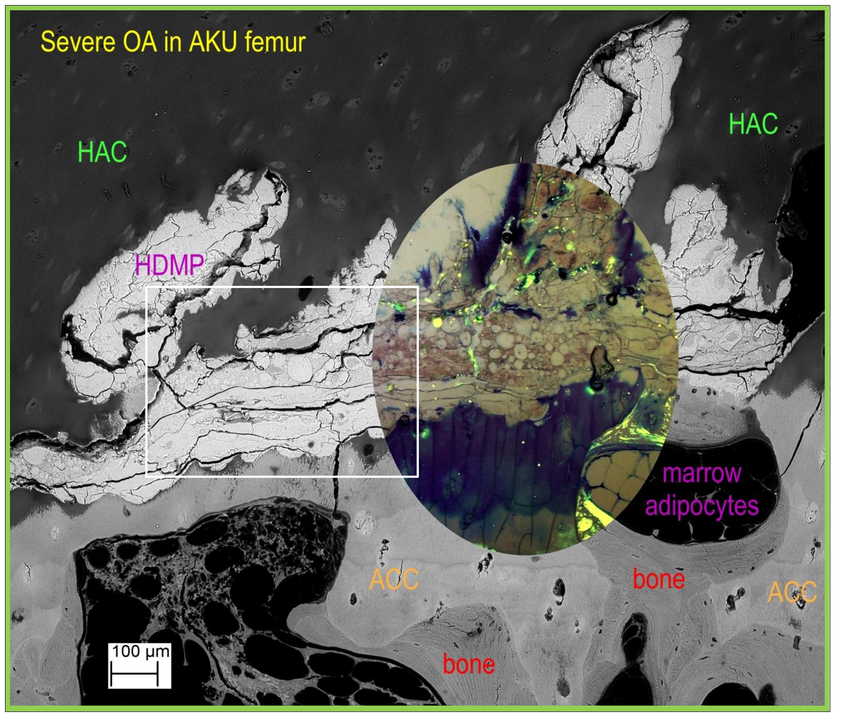
Conclusions

• LAM produces high quality, thin sections of both hard, mineralised and dense fibrous connective tissues of any sort – even single thin bone trabeculae – which can be studied with any LM method as well as BSE-SEM.
• same sample can be studied in both the SEM and the LM.
• SEM becomes extension of LM methods.
• We can perform serial thin-sectioning of the extraordinarily-difficult-to-handle HDMPs which are usually lost, flowing down the drain, with the decalcifying solutions rampant in standard histopathological laboratories, which is why they remain an unknown factor in OA.



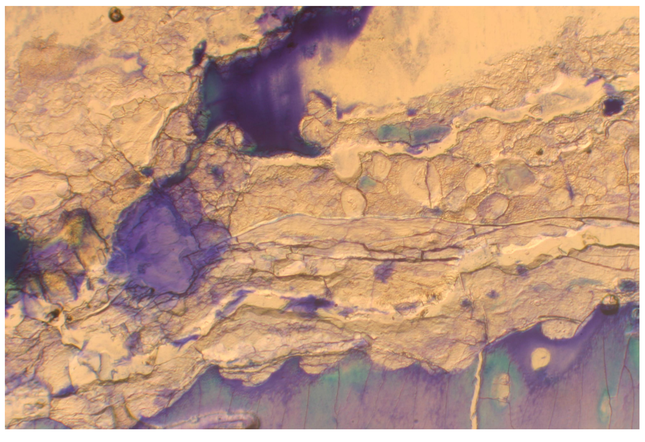
X-ray microtomography at 20 micron resolution of whole femoral head prior to any other handling procedure. Scanning time 96 hours. Showing HDMPs.

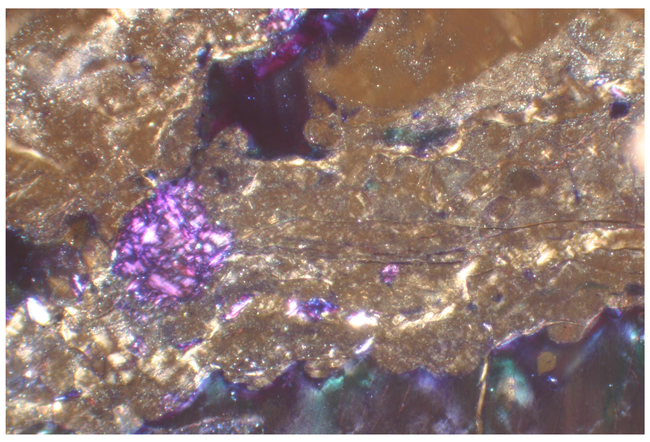
Rowiak LaserLabSolutions GmbH 'TissueSurgeon' laser microtome

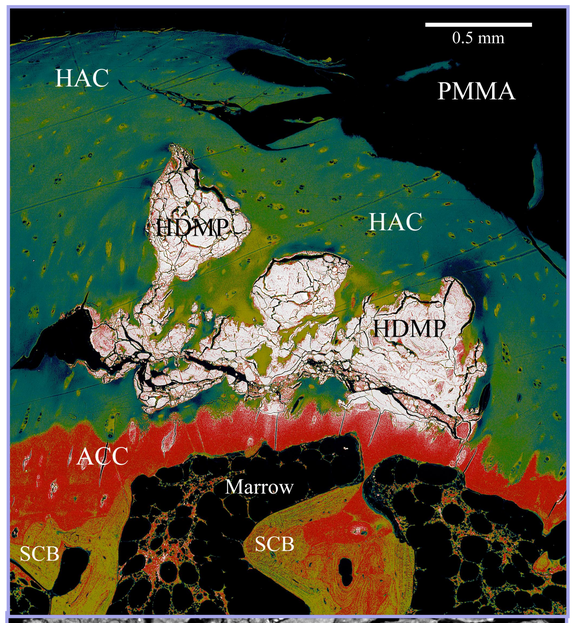


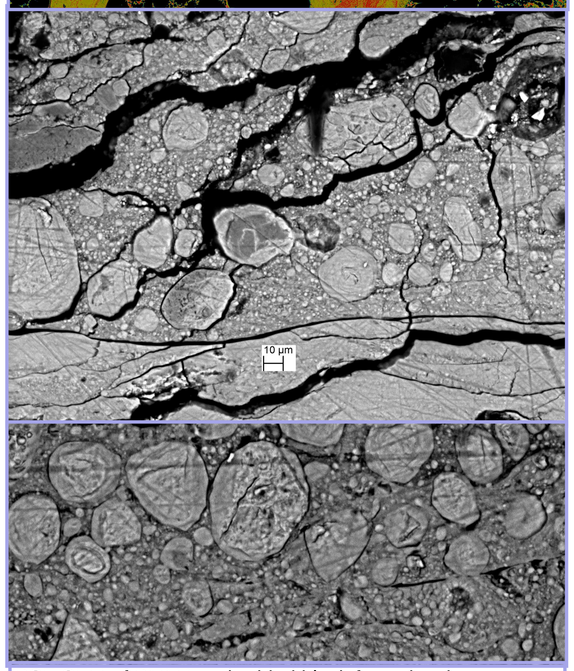
Combined Tetrachrome-stained and polarised light image within oval area, aligned and superimposed on BSE SEM image of iodine stained PMMA block face from which the 10 micron section was cut using the Rowiak 'TissueSurgeon' laser microtome.
HDMP = high density mineralised protrusion.
HAC = hyaline articular cartilage.
ACC = articular calcified cartilage.
Femoral head obtained at arthroplasty from Alkaptonuria patient who suffered severe pain from OA (case described in DOI: 10.1111/joa.12226).

Below
Detail Light Microscopy from field outlined with white rectangle above:
Left Tetrachrome staining: Right: combined with polarised light.

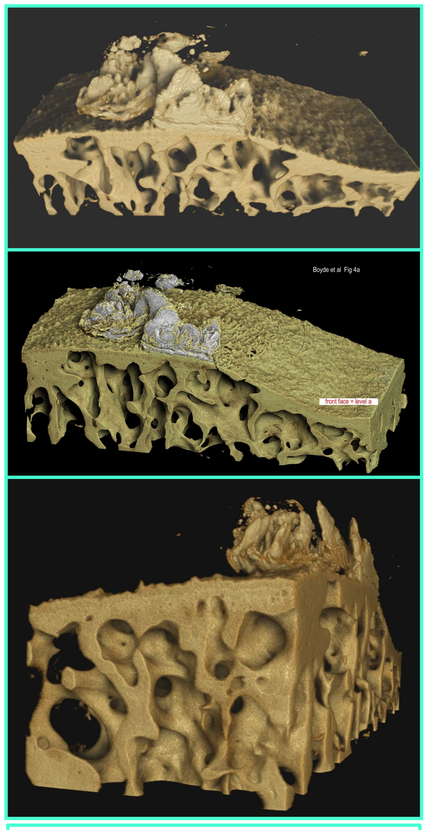








BSE SEM of PMMA embedded block face showing distribution of HDMP relative to bone, ACC & HAC and detail within HDMP, prior to LAM LM sectioning.



X-ray microtomography at 5 micron resolution of PMMA block during serial grinding and polishing for BSE SEM imaging