BSE-SEM STUDIES OF ARTICULAR CARTILAGE AND SUBCHONDRAL BONE IN NATURALLY OCCURRING POST-TRAUMATIC EQUINE CARPAL OSTEOARTHRITIS

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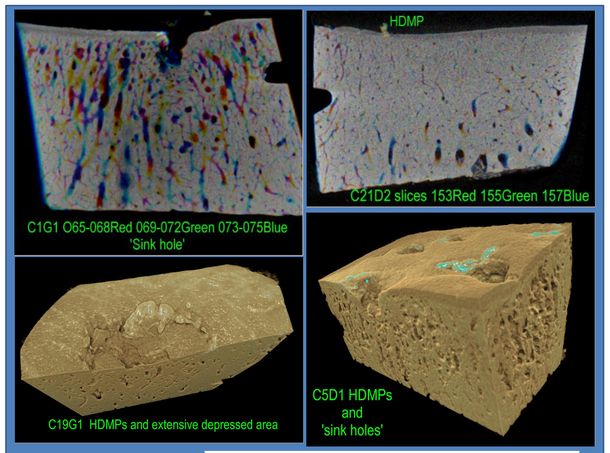
Introduction

Objectives of were to investigate osteoarthritis (OA) changes at the osteo-chondral junction in the equine third carpal bone at sites that undergo repetitive high intensity loading. Using archival x-ray micro computed tomography (µCT) data, we had previously demonstrated the presence of high density mineral infill (HDMI) in cracks in subchondral bone (SCB) and articular calcified cartilage (ACC), and extensions of HDMI to form high density mineralized protrusions (HDMPs) into hyaline articular cartilage (HAC). Here, we wished to refine this knowledge with higher resolution microscopical techniques based upon backscattered electron scanning electron microscopy (BSE-SEM).



Micro-CT

High Density Infill in Cracks and Protrusions from the Articular Calcified Cartilage in Osteoarthritis in Standardbred Horse Carpal Bones

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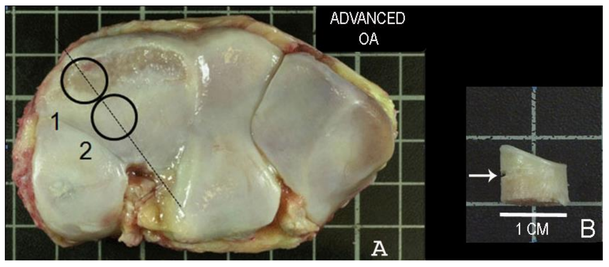
Table 1. Number of images or clusters with HDMI or HDMP spotted by code blind observer.
D = Droite = Right. G = Gauche = Left: Last digit 1 = more dorsal 2 = more palmar site.
Assignment to Control, EOA and AOA groups at post mortem acquisition of sample.

ID#	Age	number						
Controls			Early OA			Advanced OA		
C1G1	8	13	C4G1	7	0	C5D1	2	4
C1G2	8	0	C4G2	7	0	C5D2	2	0
C2G1	4	0	C5G1	2	6	C21D1	7	4
C2G2	4	0	C5G2	2	5	C21D2	7	1
C3D1	7	2	C10D2	7	4	C21G1	7	4
C3D2	7	0	C19G1	6	7	C21G2	7	0
C13G1	8	0	C19G2	6	3	C40D1	5	17
C13G2	8	0	C38D1	5	12	C40D2	5	7
C20G1	6	0	C38D2	5	0	C44D1	4	8
C20G2	6	0				C44D2	4	5

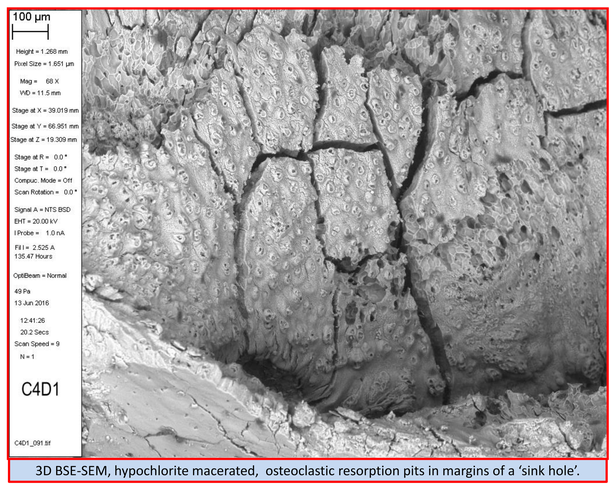
Materials and Methods

Macroscopic analysis of articular cartilage: C3 were collected post mortem from racehorses and classified into 3 groups (n = 5). **CO** (control = no lesions); **EOA** (early OA = fissures/partial thickness erosion ≤ 100 mm^2); **AOA** (advanced OA (partial or full thickness erosions ≥ 100mm^2). Osteochondral cores were drilled from a dorsal site where focal OA lesions characteristically occur **(Core 1)** and a palmar site where lesions are rarely encountered **(Core 2)**.

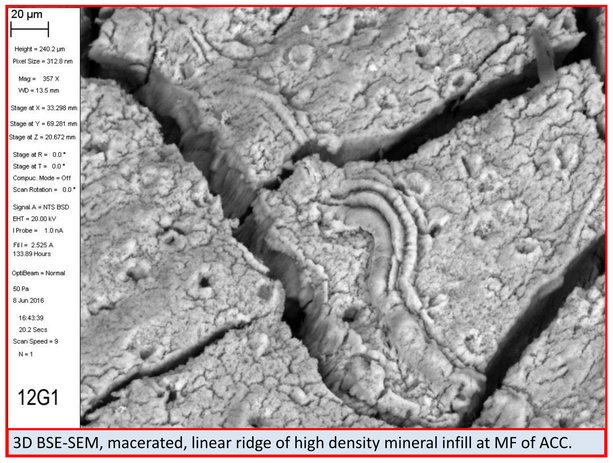


(A) Proximal articular surface of C3 from AOA group showing the location of cores.
(B) Lateral view of a core showing dorsal notch (arrow) used for orientation.

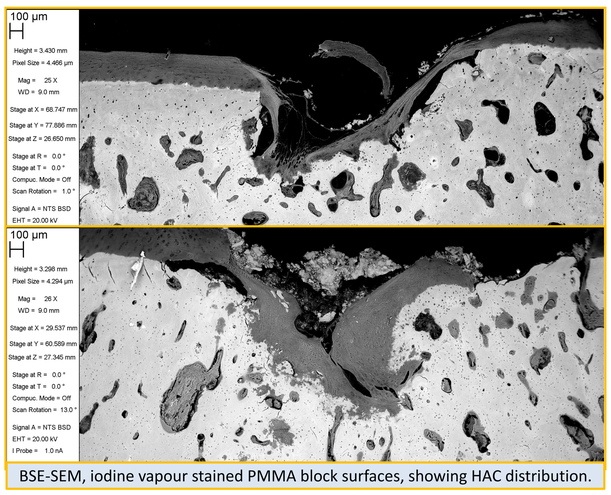
Cores were cut perpendicular to joint surface, and (A) cut surface polished and then deproteinised with sodium hypochlorite bleach to remove all cells and non-mineralized tissue, washed, dried and (i) examined by 3D BSE-SEM: then (ii) embedded in PMMA before re-cutting and polishing the block surface for 20kV BSE-SEM to provide mineral concentration dependent imaging: or (B) directly embedded in PMMA before (iii) cutting and polishing the block surface prior to staining with iodine vapour for BSE-SEM imaging of both mineralized bone and cartilage and uncalcified osteoid and cartilage: (iv) blocks were re-polished to remove the iodine stained layer to permit mineral concentration dependent BSE-SEM imaging, this last step being repeated several times to expose new section planes, and (v) then transversely to produce en face views.



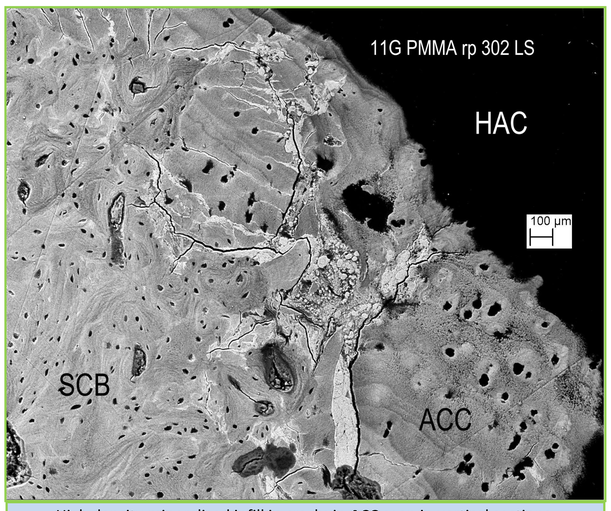
3D BSE-SEM, hypochlorite macerated, osteoclastic resorption pits in margins of a 'sink hole'.



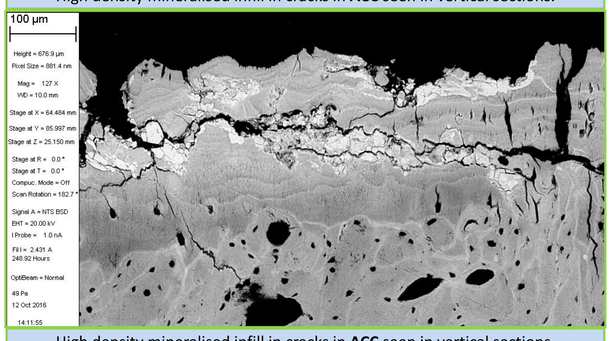
3D BSE-SEM, macerated, linear ridge of high density mineral infill at MF of ACC.



BSE-SEM, iodine vapour stained PMMA block surfaces, showing HAC distribution.



High density mineralised infill in cracks in **ACC** seen in vertical sections.



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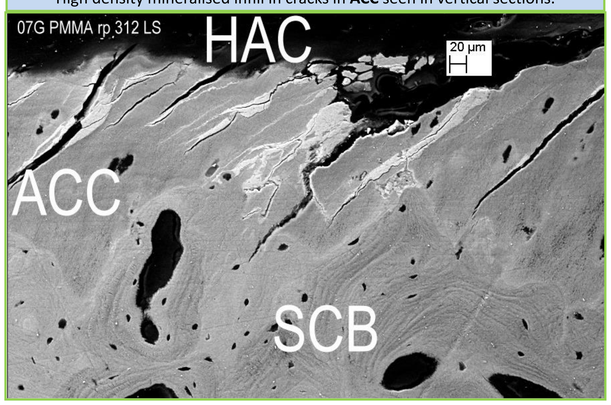

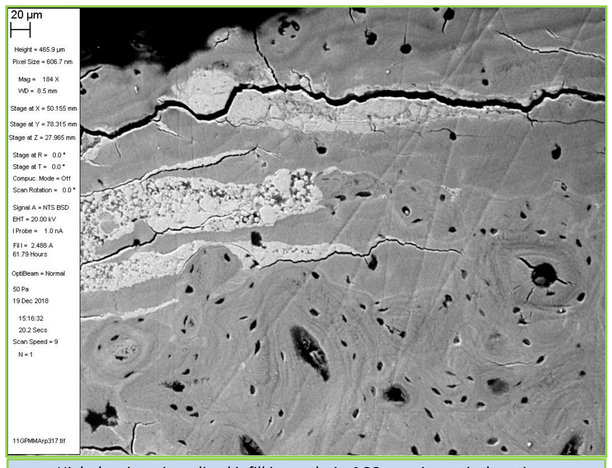

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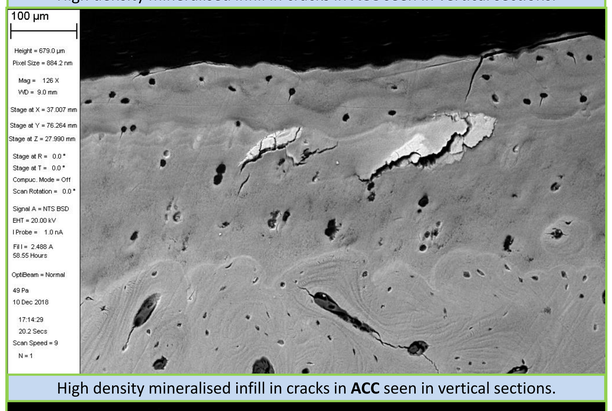

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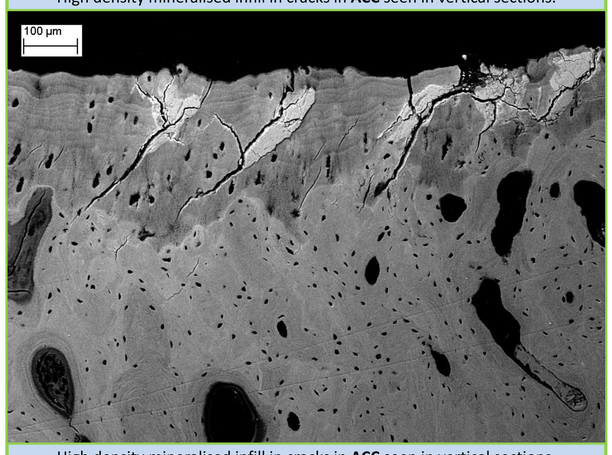
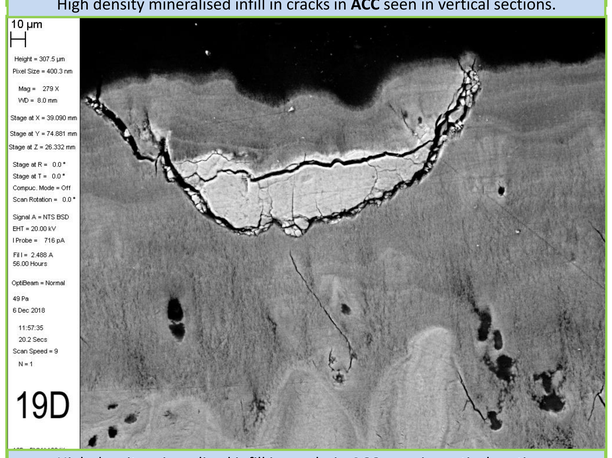
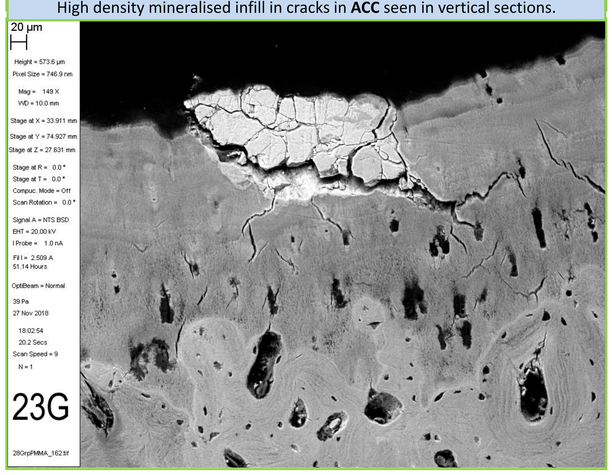
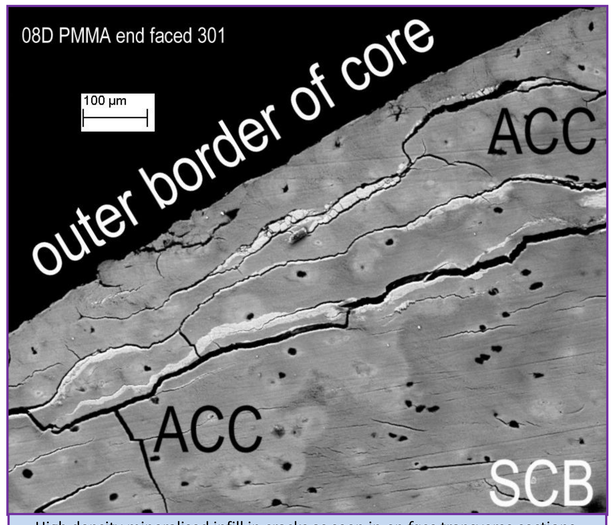


High density mineralised infill in cracks in **ACC** seen in vertical sections.

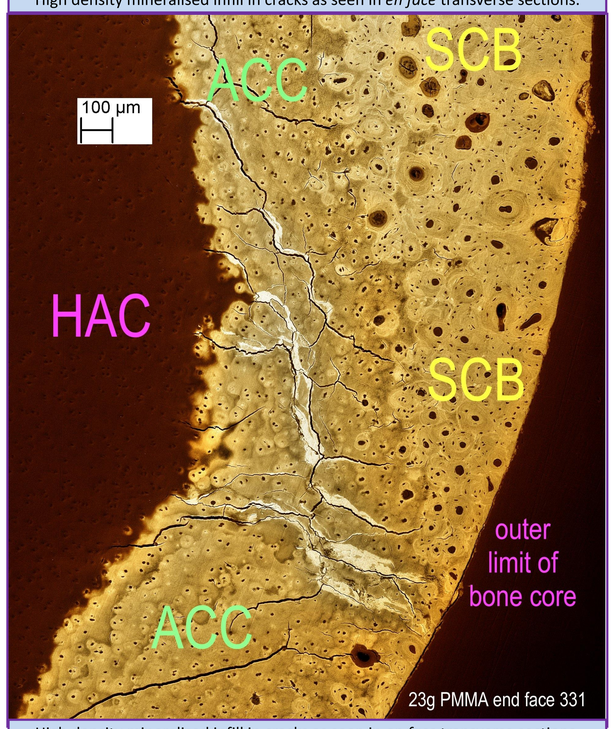


High density mineralised infill in cracks in **ACC** seen in vertical sections.

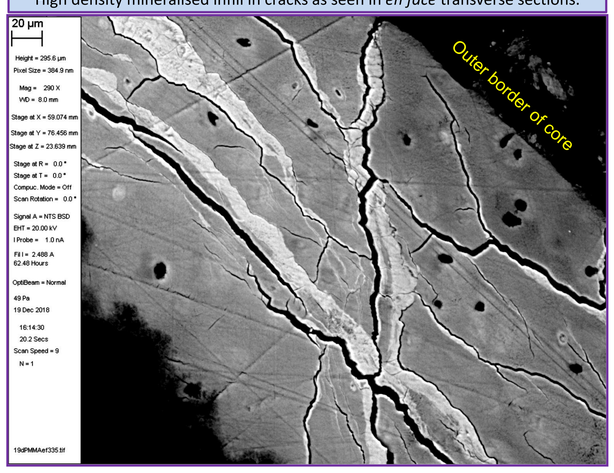




High density mineralised infill in cracks as seen in *en face* transverse sections.



High density mineralised infill in cracks as seen in *en face* transverse sections.



Summary and Conclusions

- Cartilage patches were sometimes observed deep within the subchondral plate.
- 'Sink holes' - depressions in the (tidemark) mineralizing front (MF) of the ACC - showed extensive (dissecting or undermining) osteoclastic resorbtion of both of ACC and SCB.
- All cases with overt OA had HDMI in linear cracks in ACC and SCB as had been previously suggested by micro-CT.
- Some low profile HDMPs extended from the ACC mineralizing (tidemark) front into HAC.
- A frequent feature was the obliquity of the HDMI filled cracks, at 45 or other angle to the ACC MF surface rather than perpendicular to that surface and parallel with the main collagen fiber orientation.
- We found the de novo acellular deposition of HDMI and HDMP using all three BSE-SEM modes employed. These little-known features disappear with tissue decalcification in routine histology because of their predominant mineral content.
- HDMI mends cracks.
- HDMPs are contenders for mechanical tissue demolition in OA.