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Combined novel approaches to the microscopic study of the dental implant site

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MATERIAL
We studied trephine core samples taken from 252 sites where implants were to be placed in a series of 35 patients from KW's practice: all material was obtained with patient consent and local ethical committee approval. The sites were both 'native cores' - where implants were placed at the first operation - and grafted sites, where a sinus graft procedure had been performed approximately six months before the second operation to place the implant. The initial studies were performed in 1996-1999 while both KW & AB were at UCL [1].

[1] Wong K. Studies of the quality of the intraosseous dental implant bed and of thermal effects in implant pathology. PhD Thesis, 2000, University College London, 190pp.

METHODS (1)
Samples were fixed in 70% ethanol, dehydrated in ethanol and embedded in PMMA. Resulting blocks were finished to produce polished longitudinal sections through the cores. Real time direct view 3D fluorescence light microscopy was used to study tissue morphology before carbon coating for study by qualitative and by quantitative backscattered electron (qBSE) scanning electron microscopy (SEM) imaging using brominated and iodinated dimethacrylate standards to calibrate backscattering (mineralisation density) [2].

For further correlative light microscopy (LM) for cell and uncalcified matrix histology, blocks were lightly repolished to remove the carbon coat, surface stained with toluidine blue and ethidium bromide, and studied using transmitted light and/or the background fluorescence of the sample to illuminate the stained surface layer.

[2] Boyde A, Travers R, Glorieux FH, Jones SJ (1999) The mineralisation density of iliac crest bone from children with osteogenesis imperfecta. Calcified Tissue International **64**:185-190.

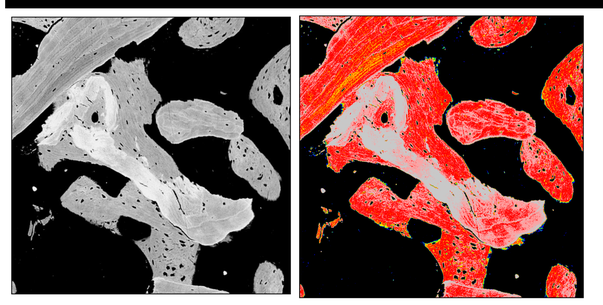
METHODS (2)
Later (in 2000 and 2001), several cores were studied by x-ray microtomography (XMT/µCT) using the QMUL MuCAT scanner. This enabled us to make direct correlations between the current block face imaged with qBSE SEM and rendered XMT data.

We revisited the same blocks (2011-2015) after evolving iodine staining methods for BSE SEM of PMMA block surfaces at 50Pa chamber pressure [3,4]. This enabled us to read the soft tissue and cellular histology directly in the SEM image of uncoated blocks and represents an important advance in the utilisation of archival resin embedded histological material in implant related research.

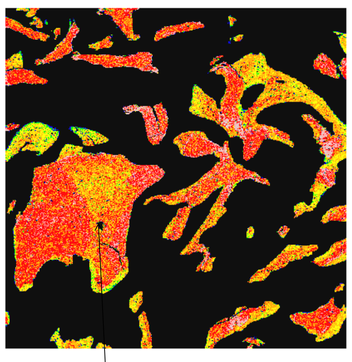
[3] Boyde A. Staining plastic blocks with triiodide to image cells and soft tissues in backscattered electron SEM of skeletal and dental tissues. Eur Cell Mater. 2012 **24**:154-60.

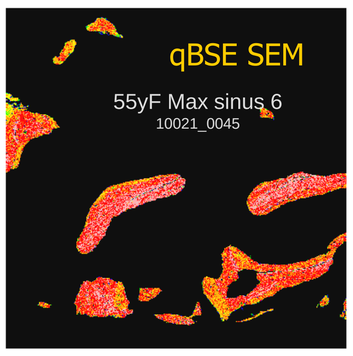
[4] Boyde A, McCorkell FA, Taylor GK, Bomphrey RJ, Doube M. Iodine vapor staining for atomic number contrast in backscattered electron and X-ray imaging. Microsc Res Tech. 2014 **77**:1044-51.

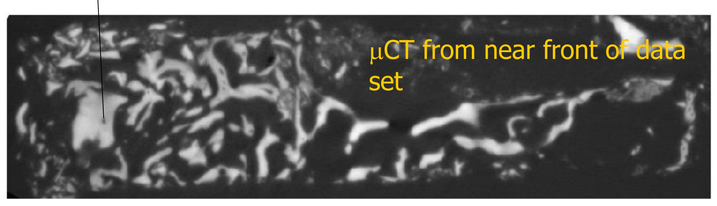
Recent extraction socket kwd63

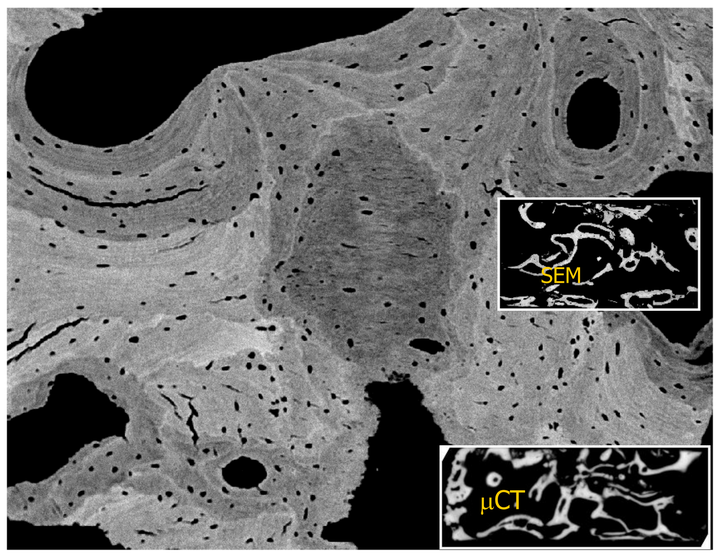


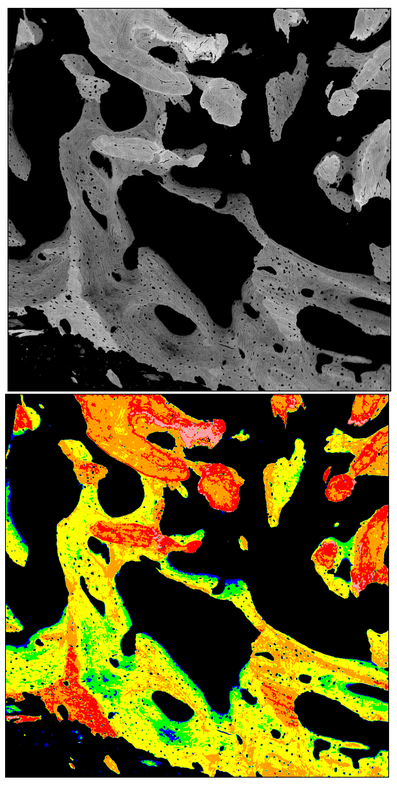
- bone mineralisation density from BSE-SEM
- >1000-fold better volume resolution than microradiography or microtomography
- established routines for quantification
- tissue fixed in 70% ethanol > ethanol > PMMA
- block trimmed and polished, front parallel back
- conductive tracks around rim of milled face
- coat with carbon
- 80mm * 80mm specimen arrays
- digital SEM, 20kV and 0.5nA
- annular solid state BSE detector

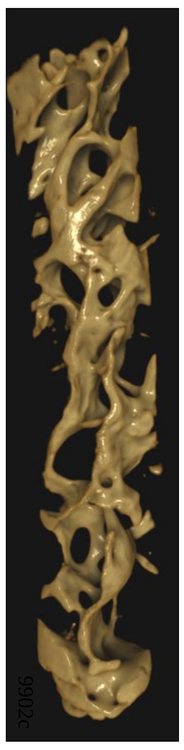


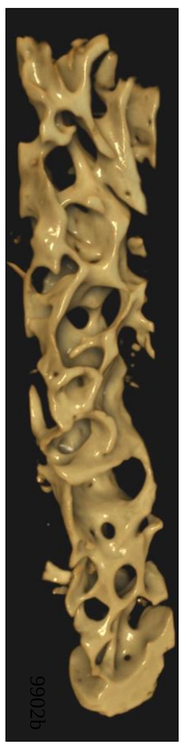


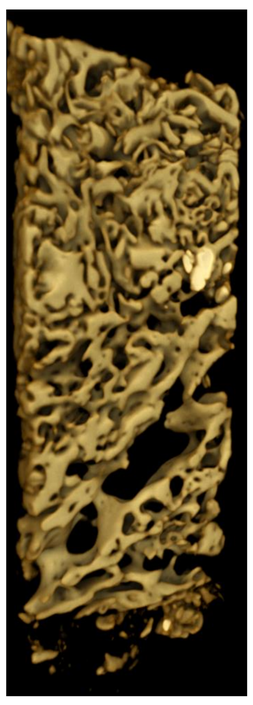


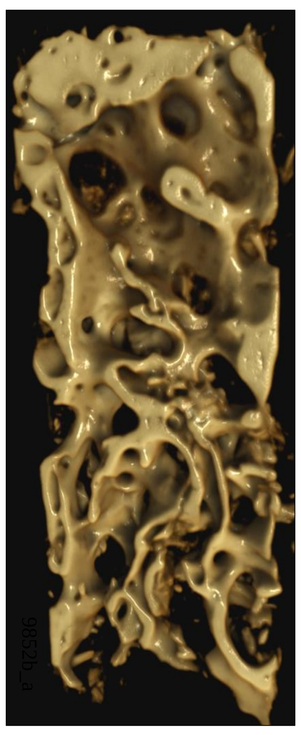


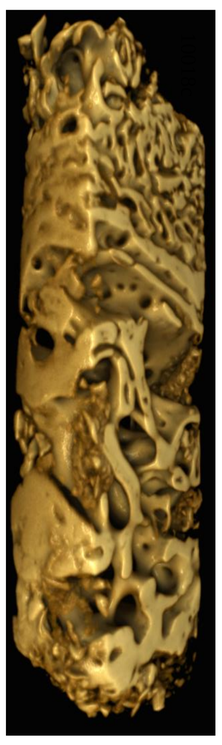


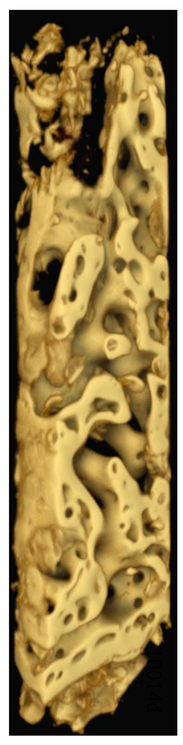


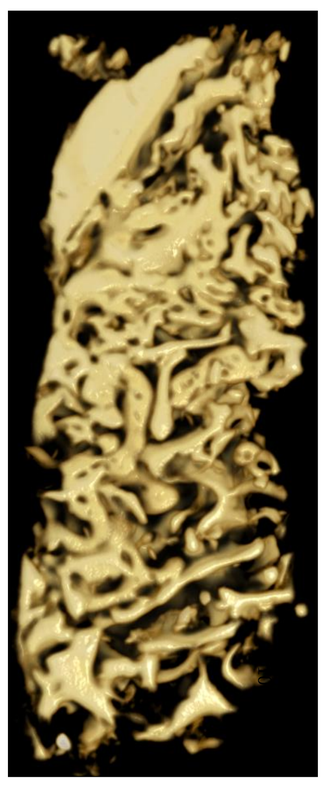


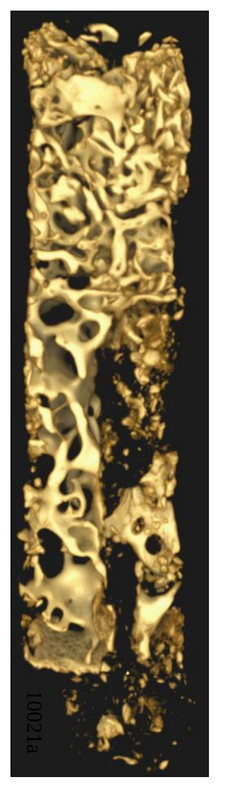


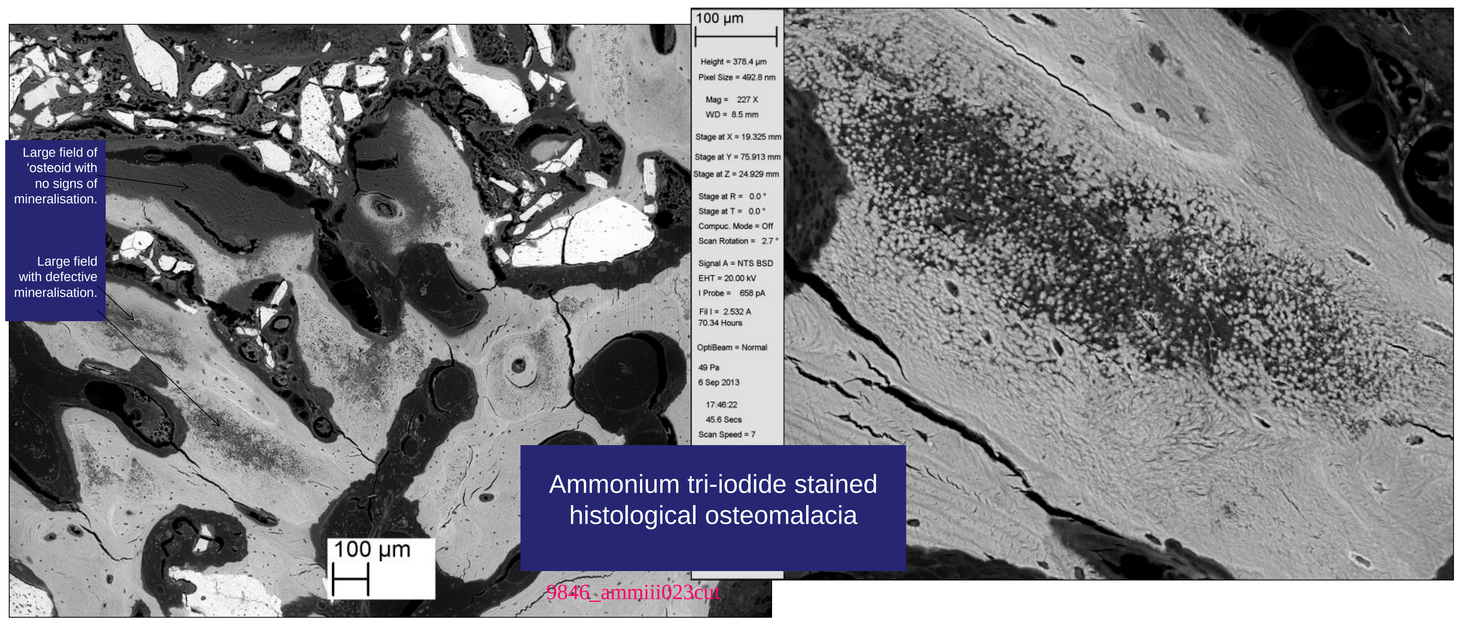


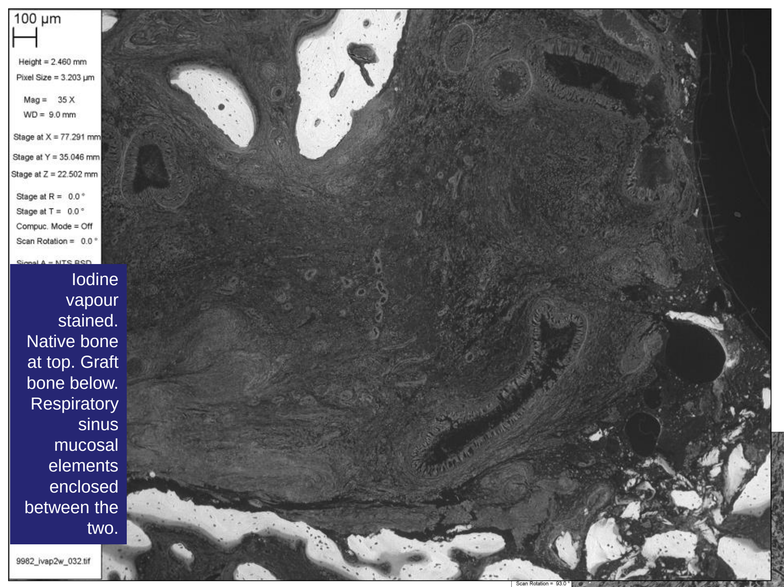


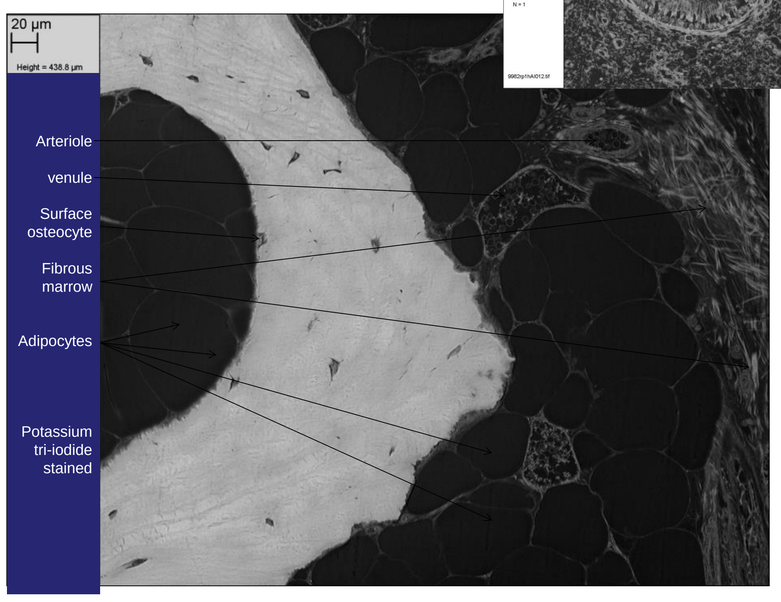












Summary and conclusions

- Valuable information can be retrieved from the study of the bone core removed to make the space for the dental implant.
- The bone core can be studied asap, or stored for later analysis, eg in 70% ethanol.
- Derived data could be used for research or medico-legal or forensic evidence.
- Do not give the core to the 'histology lab', since they will hardly be able to constrain themselves from demineralising the sample, when half the evidence goes down the sink.
- µCT can be done on the wet retrieval core with no further processing.
- the moderate spatial resolution available with standard lab µCT systems will suffice both to study the connectivity and volumetric density of native bone, and the interconnectedness of graft bone with patient's new bone.
- The main function of the live osteocyte is to prevent or down-regulate bone mineralisation: dead bone, including all grafted bone accumulates mineral and will be recognised as a denser phase.
- Block face methods are strongly preferred for this kind of sample: it should be embedded in a resin: we have found PMMA to be satisfactory: sections as such contain only damaged tissue: the intact tissue remains in the block face, which can be finished by diamond micromilling (expensive) or polishing (cheap).
- Microscopic methods for studying the block face include reflection and fluorescence conventional and confocal optical microscopy, but VIP BSE SEM: iodine or tri-iodide staining permits the study of soft tissue elements including cells and matrix.
- A variety of bone types are found in the retrieval core samples, in various mixtures, and it is not satisfactory to say that there is, or is not, bone there.
- Bone varies widely in its mineral content (concentration) and this is best studied by qBSE SEM since µCT does not have sufficient spatial resolution; LM, qBSE SEM, Iodine contrasted BSE SEM and µCT are easily correlated.
- Most bone in the jaw bone wound site – which is what is studied in the graft retrieval cores – contains a high proportion of large diameter collagen fibre bundles (Sharpey fibres) which are also extensively present in the surrounding marrow spaces: these are only incorporated in normal bone at periosteal sites and in the bundle bone of the socket, where PDL Sharpey fibres are inserted.
- Direct lining of internal bone surfaces with adipocytes is only sometimes found in native cores, whereas post-cranial bone sites usually have adipocytes as the lining cells in older mature bone.
- Sinus graft cores sometimes include respiratory epithelium and associated glandular epithelium: we are not aware that this has been reported previously, though it is an obvious risk.
- A high frequency of arterioles may be found in the 'marrow' space in sinus graft retrieval cores: small blood vessels may be in extremely close proximity to - or in contact with - bone.
- Bone mineral content and osteomalacia: more research needed.
- Substantial regions of non-mineralised bone matrix (osteoid) are found in some retrieval cores, easily spotted in iodine contrasted SEM: some such contain no mineral, and others small unfused mineral patches.
- Recognition of osteoid is simple in iodine contrasted SEM.