Correlation between 3D imaging methods in studying bone architecture: SEM, microCT and confocal LM

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Computerised x-ray microtomography (µCT) is increasingly used in the 3D study of bone microarchitecture and in quantifying bone volume fractions. However, the volumetric resolution in laboratory apparatus for small rodent studies is at best several microns linear and recognisable detail characterising forming, resting and resorbing surfaces is completely missing. Backscattered electron mode scanning electron microscopy (BSE SEM) of both macerated 3D samples and polished surfaces of blocks of PMMA embedded tissue provides this information but samples have to be cut and processed. The same PMMA material is good for confocal fluorescence microscopy (CSLM) for both tissue morphology and the study of tetracycline and calcein mineralising front labels to 50–200 microns deep to the block surface. With the recent acquisition of SEM with variable chamber pressure to permit examination of uncoated specimens, we are able to conduct CSLM after SEM for correlation studies. Here, we report new approaches to correlation between all these imaging methods in the study of 70% ethanol fixed normal femurs from ~ 330 g male Lewis rats from another study. Microtomography used a Scanco µCT 40 system using 45 or 55 kV and 8 µm linear voxel size. The distal femur was bisected longitudinally with a water cooled diamond saw, one half was macerated in alkaline pronase and the other embedded in PMMA prior to 20 kV 3D BSE imaging. Embedded block faces were imaged uncoated in the SEM and by confocal microscopy. Volumetric data analysis used ImageJ. Drishti software (Australian National University) was used to reconstruct views corresponding to the SEM images with particularly good matches between SEM and µCT for the macerated trabecular bone. Extensive regions of thin trabeculae were frequently missing in the µCT reconstruction. This is clearly a partial volume problem but it draws attention to the fact that an extensive network of fine trabeculae are lost to µCT visualisation and analysis on a routine basis. These fine rods are frequently ~ 7 and down to < 2 µm in gauge, well below the detection limit for µCT. Their existence overturns results and theories about interconnectedness.

Characterisation of the ossified avian tendon

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