Investigation of the 3D vascular network in vertebral end-plates of mature sheep using micro-CT

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Aims

Back pain has been found to be the leading cause of disability worldwide, affecting 8 out of 10 adults (1). One of the major causes of back pain has been identified to be intervertebral disc (IVD) degeneration. The disc being the largest avascular tissue in the body, it relies primarily on the nutritional pathways from the adjacent vertebral endplates (VEPs) (2).

The VEPs are characterised as a bilayer of cartilage and bone, which acts as a boundary between the IVD and the vertebral bone (3). Previous work done by Lotz et al. has shown the presence of an intricate network of vascular canals in the VEP through which nutrients are carried to the IVD and waste products are removed (4). However, the complete structural characterisation and quantitative evaluation of these canals throughout the spine are yet to be carried out.

The aim of this work is to carry out qualitative and quantitative assessment of the 3D vascular network in the VEP, in the first instance and to then investigate the effect of the position of the VEP along the spine and degree of degeneration of the disc on the canal network.

Method

It has been shown that sheep spines provide the most reliable model for human spines (5). Ideally, sheep spines are fully developed when the sheep is 3 years or older. Therefore, spines from mature sheep of about 36 months old were preferentially used. These spines were collected from the animal farm of the Division of Trauma and Orthopedic Surgery, Cambridge University and frozen at – 20 °C until used.

The spines were thawed in the fridge overnight before use. Using surgical scalpels, an incision was made through the middle of the intervertebral disc to separate the two adjacent vertebrae, as shown in Figure 1.

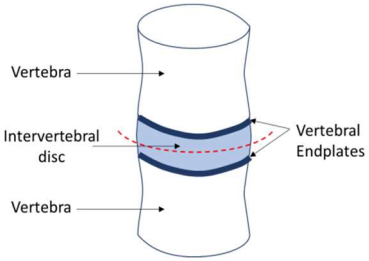

Figure 1: Schematic diagram showing where the incision was made (along red dotted line) across the disc to separate the two adjacent vertebrae

Using a 115 mm long trephine of 5 mm outer diameter and 3 mm core diameter from

Veterinary Instrumentation (Sheffield, UK), cylindrical rods were punched out of each segmented vertebra. Each rod was 3 mm in diameter, approximately 10mm in length and with the VEP sandwiched between a layer of disc and the underlying trabecular bone, as shown in Figure 2. Samples were collected from the central and peripheral regions of the VEP.

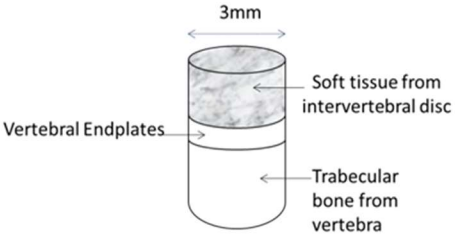

Figure 2: Schematic diagram showing trephined cylinder with the VEP between the disc and the vertebra

Samples were scanned in wet conditions (using phosphate buffer saline) using a Skyscan 1172 Micro-CT with X-ray voltage 65 kV and current 153 µA. Samples were centered carefully on the stage within the apparatus. Before scanning, flat-field correction was carried out to remove artefacts caused by variations of the pixel-to-pixel sensitivity of the detector. All scans were taken with the camera in the medium range, with a pixel size of 4.92 µm, an angular step size of 0.4 ° and exposure time of 7.5 seconds. A 0.5 mm aluminium filter was used during the scan to optimise transmission. Reconstructions were carried out using the software, NRecon. Thresholding was kept constant (minimum: 0, maximum: 0.1) for all the scans to ensure reliability for comparison purposes. DataViewer was used to view and manipulate the reconstructed images in 3D orthogonal views.

ImageJ and ScanIP (Simpleware, Synopsys Inc., USA) was then used to create 3D rendered volumes of the canal network found in the VEP using the reconstructed micro-CT images using segmentation and flood fill to isolate the vascular network. Centrelines were added and enabled the quantitative analysis (diameter, length, orientation and connectivity) of individual canal.

Results

Micro-CT images confirmed previous work by Pitzen et al. that VEP samples extracted from the central region and the peripheral region of the VEP exhibit differences in terms of thickness and porosity (6). Figure 3 shows thinner VEP layer with a greater concentration of pores at the upper surface whereas Figure 4 shows a thicker layer of VEP with fewer pores. The average thickness of the 5 samples from the central region was measured at three points per sample as 0.57 ± 0.18 mm and 1.22 ± 0.23 mm for peripheral region samples. The trabecular structure in both types of samples was found to be similar.

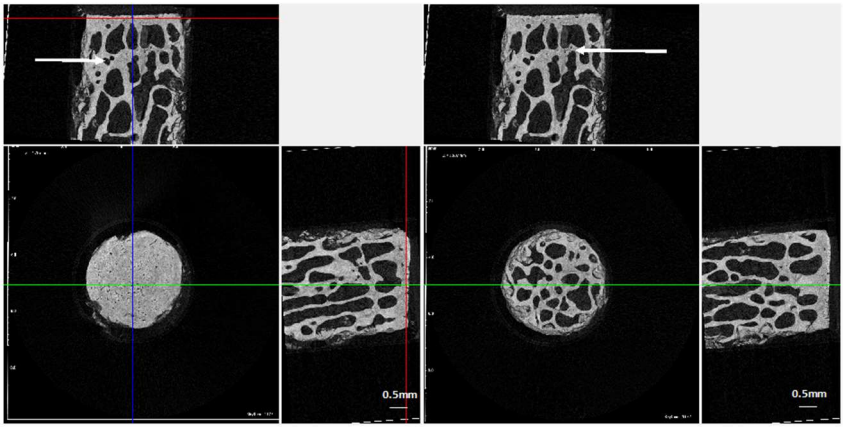

Figure 3: Micro-CT image of mature VEP from central region showing VEP (left) and trabecular structure of vertebra (right), with white arrows representing epiphyseal layer

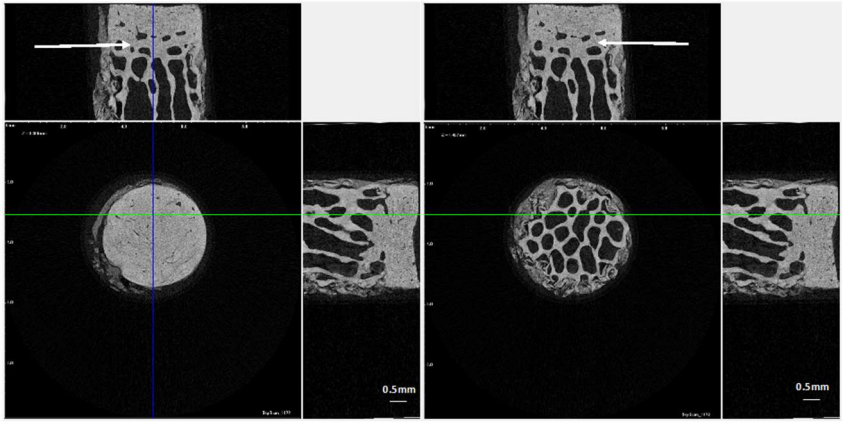

Figure 4: Micro-CT image of mature VEP from peripheral region showing VEP (left) and trabecular structure of vertebra (right), with white arrows representing epiphyseal layer

Image J was used to create 3D rendered volume images of the micro-CT data as shown in Figure 5. Simpleware was then used to further analyse the intricate 3D canal network observed in the VEP, as shown in Figure 6.

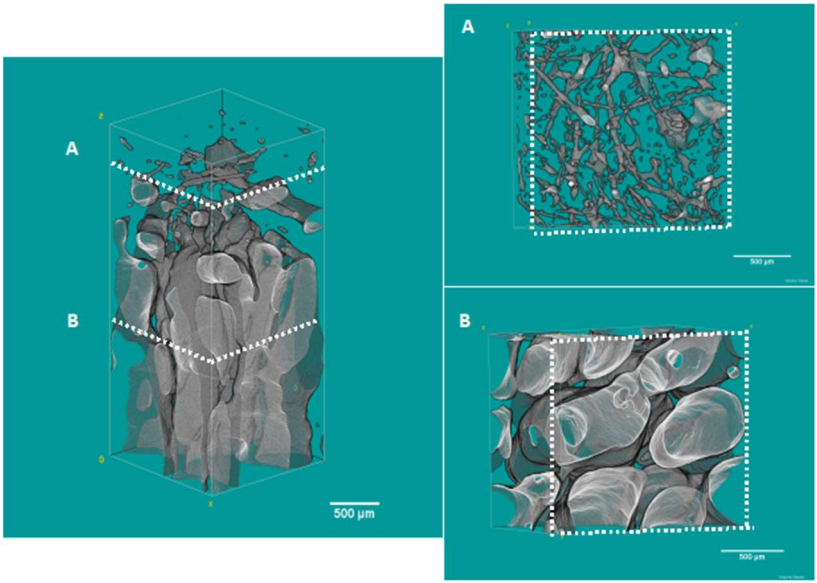

Figure 5: 3D rendered images of a volume of interest in central region sample. Left: vertical section of sample showing transition from sponge-like trabecular (B) to less dense VEP (A).

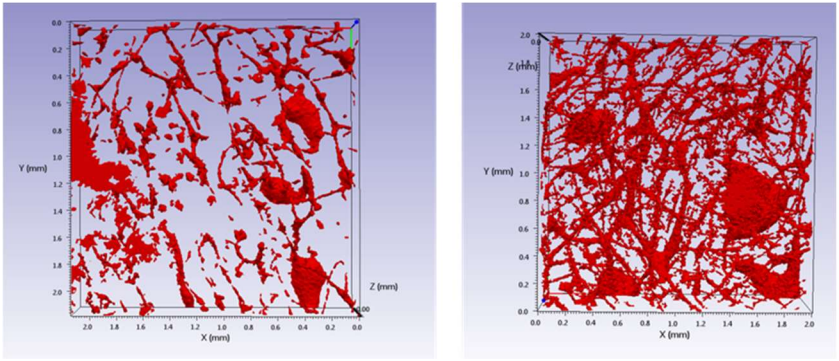

Figure 6: 3D rendered images of one volume of interest from central region (right) and one from peripheral region (left) of the VEP

It can be observed that there are considerably more branched out canals in the central region, below the nucleus pulposus of the intervertebral disc. Using the centreline editor in ScanIP, it is possible to isolate individual continuous branches and extract quantitative data as shown in Figure 7 and Figure 8.

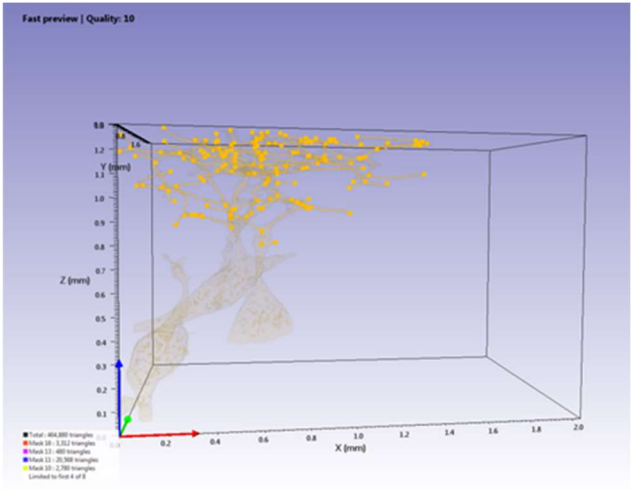

Figure 7: Isolated branch with centrelines and nodes added

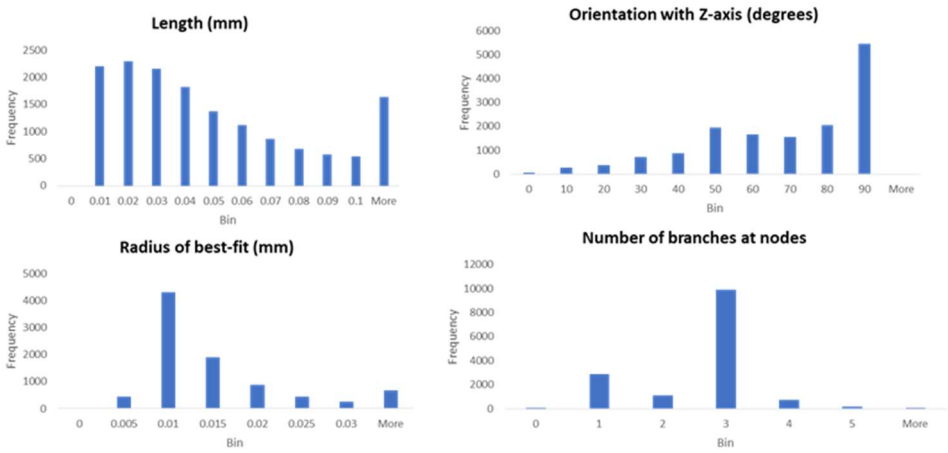

Figure 8: Measurement of the distribution of length, angular orientation with the Z axis, radius of best fit and number of branches at nodes of each branch in one sample

Conclusion

This work demonstrates that the micro-CT, coupled with ScanIP is an extremely useful tool for the characterisation and quantitative analysis of the intricate 3D vascular network in the VEP. The next steps will be the comparison of the network in the healthy VEP and in VEP adjacent to degenerated discs to understand the relationship between disc degeneration and the nutritional pathway to the disc.

References:

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