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Investigations of vascularisation and blood flow at the subchondral plate using an X-ray fluorescence technique

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1. Introduction

Articular cartilage is avascular and conventional dogma is that it receives its nutrition from synovial fluid via the articular surface. Although there is a rich blood supply in the subchondral bone it is believed to be separated, in the mature animal, from the cartilage by an impermeable end-plate. In re-examining this view it has become apparent that quantitative data on blood volume and flow in the subchondral region are extremely scarce. This is no doubt in part due to the difficulties in studying bone using standard techniques of flow measurement such as radio- or fluorescent-labelled microspheres or laser Doppler flowmetry and even in processing for histology. We have, therefore, sought to develop an X-ray fluorescence method that overcomes these difficulties. The technique can also be combined with synchrotron micro small-angle X-ray scattering (μSAXS) or micro wide-angle X-ray scattering (μWAXS) methods of structure determination, allowing local blood flow to be related to tissue architecture.

2. Experimental method

Flow measurement was based on a modification of the familiar microsphere method (Prinzen and Glenny, 1994; Nakajima et al., 1999; Prinzen and Bassingthwaighte, 2000). Equine fore-limbs were obtained from the abattoir. Immediately after slaughter the external medial artery was cannulated and approximately 0.5 l of 0.15 M NaCl were flushed through until the drain from the contralateral vein was free of blood. The limb was then rapidly transferred to the laboratory and perfused at an arterial pressure of 120 mmHg. When flow had stabilised, 4 ml of silver-coated microspheres (Ag content ~13% of total weight of microsphere; BioPhysics Assay Laboratory, Inc, Worcester, MA) 15 μm in diameter were introduced into the inflow stream over a known time period. After 2 min the saline perfusion was stopped and the bone was dissected from surrounding tissue and immersed in 4% formal saline. For blood content measurements the limb was plunged into fixative immediately after dissection. In both cases sections 1 mm thick were sliced longitudinally and parallel to the long axis of the limb using a band saw. Several sections were also cut perpendicular to the same axis.

The cut section was mounted on the sample stage of the ESRF beamline station ID18F, at an angle of approximately 45° to the incident beam. Energy dispersive X-ray fluorescence measurements were made with a beam size of 50 μm and acquisition time of 10 s. Using a scanning stage an 60 μm × 60 μm array of measurements were acquired over a field of up to 6 mm × 6 mm. Using an excitation energy of 27 keV, the silver K_α fluorescence was detected at 22 keV. Blood content was measured from the K_α fluorescence of iron (6.40 keV) using either the synchrotron source or a Cu tube X-ray source. To investigate the organisation of collagen and mineral in the same field both small-angle X-ray scattering and wide-angle X-ray scattering measurements were performed.

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3. Results and discussion

The detection of silver fluorescence was sufficiently sensitive to allow the detection of single particles. A typical intensity plot for a normal region of tissue is shown in Fig. 1. The absence of flow above the calcified zone is immediately apparent and the distribution of fluorescence correlates well the capillary density observed in histological sections. Whether the variations in intensity represent multiple particles in a single vessel or juxtaposed capillaries remains to be established. Indeed, the spatial resolution evident in the figure has not been reported in previous microsphere studies and raises further questions about the mechanisms of microsphere trapping that have not been addressed.

Determination of capillary blood volume from the fluorescence of iron was even more sensitive, due to the high concentration of iron in haemoglobin. Satisfactory data could be obtained even with a Cu tube source. With the synchrotron extravasated blood associated with bone lesions could be assayed. In normal tissue there was no evidence of poorly perfused capillaries at the bone–cartilage interface.

In addition to its sensitivity, another major advantage of the technique is that features of tissue structure can be identified in the same tissue specimens by fluorescence or diffraction. In bone and cartilage the most informative indices are calcium crystal and collagen fibre structure and organisation.

4. Conclusion

Investigations have been made on the microvasculature of subchondral bone in the equine metacarpus. Perfusion was measured using X-ray fluorescence of silver-doped 15 μm microspheres in 1 mm thick sections. This approach avoided the difficulties in processing calcified tissues for radioactive- or coloured-microsphere measurements. It also provides high sensitivity and spatial resolution. Microcirculatory blood volume and, in pathological tissue, extravasated blood was measured using haemoglobin fluorescence. These measurements can be combined with microdiffraction analysis of tissue structure and, together these techniques provide a new approach to the analysis of relationships between blood flow and tissue pathophysiology.

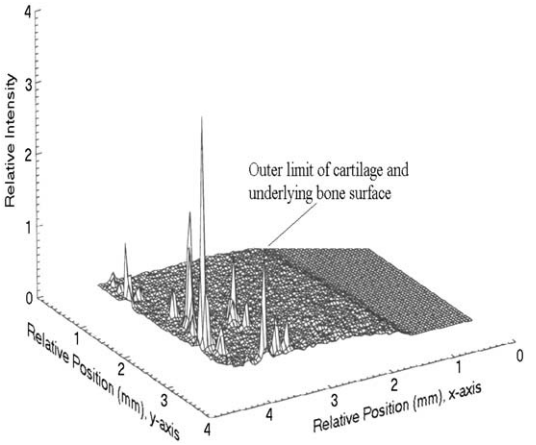

Fig. 1. Raster scan, showing detection of K_α fluorescence from the 15 μm Ag spheres, lodged some 1.5 mm below the cartilage surface.

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