

THREE DIMENSIONAL MULTISCALE RECONSTRUCTION OF EMU FEMORAL HEAD OSTEONECROSIS: FROM CELL TO ORGAN LEVEL

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INTRODUCTION

Osteonecrosis of the femoral head is a disorder whose mechanical pathogenesis (collapse) is significantly affected by the geometric details of the necrotic lesion. The (bipedal) emu model of osteonecrosis, unlike other animal models, mimics human clinical collapse. For lesions induced by liquid nitrogen insult, systematic studies of the resulting structural compromise require delineation of zones of necrosis in three dimensions relative to the global femoral head. Unfortunately, the gold standard for determining osteonecrosis is by time-consuming histologic evaluation, thus requiring novel multiscale analysis.

Automated histologic image analysis [1], which can determine osteocyte viability, requires that global orientation of the femoral head be preserved throughout histologic processing and analysis. Post-analysis, data from multiple slides need to be reassembled into a three-dimensional global map of osteocyte survival.

METHODS

Immediately post-necropsy, the proximal portion of emu femur is fixed in formalin. After fixation, the femur is landmark leveled and potted upright in PMMA. The potted specimen is then transferred to a custom drill guide and two parallel holes, 1.6 mm in diameter and 10 mm apart, are drilled through the femoral neck (Figure 1). Two orthogonal cuts are made 3.8 mm from the holes to remove the femoral head and neck from the remainder of the proximal femur. The femoral head is then decalcified in formic acid.

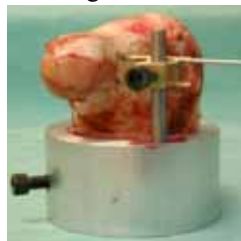


Figure 1: Potted femur in drill guide.

Since there is no practical way to preserve serial section registry after paraffin embedment and conventional microtoming, it was necessary to introduce fiducial markers that had the unique attributes of accurate shape preservation during subsequent histological processing, yet sufficient friability to not damage the delicate microtome blades. After investigating many candidates, we found that ordinary household potato had that combination of properties.

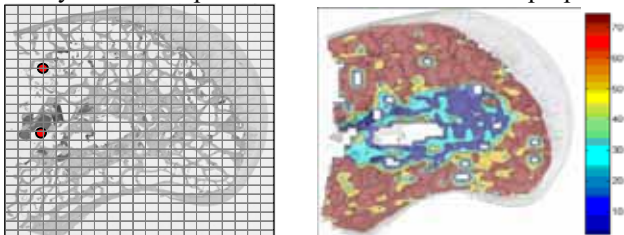


Figure 2: (a) Reconstructed slide image with dowel marker (red) and grid lines between every second subimage. (b) Two dimensional contour plot of osteocyte survival.

Upon decalcification, two 1.5 mm diameter dowels of potato, dried for 48 hours in a 42°C oven, are inserted through the two holes in the femoral neck. The femoral head, with fiducial dowels in place, is then dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Serial sections are taken every 0.5 mm through the entire thickness of the head. Each section is mounted and stained in Wiegert's hematoxylin and eosin.

Each slide is scanned on a stepper-motor-driven microscope stage, and saved in the form of approximately 2000 subimage .tif files. Every subimage is analyzed to measure fractional osteocyte survival. These individual subimages are then reconstructed in Matlab into a whole-section image, and osteocyte viability data for each slide are reassembled into a corresponding two-dimensional matrix (Figure 2). Each two-dimensional whole-head section is rotated and translated until the centroids of its two dowel fiducial markers are coincident with those from the other sections (Figure 3). After this affine transformation, these sections are assembled into a three dimensional matrix, and interpolated to output the three-dimensional distribution of osteocyte necrosis.

RESULTS AND DISCUSSION

The present three-dimensional arrays of fractional osteocyte viability represent the first instance of histology-based osteocyte status being mapped at the whole-bone level. This allows direct registration of the cryo-induced lesion relative to osseous stresses that result from external loading of the joint, toward the goal of understanding the role of lesion pathogenesis in global mechanical collapse.

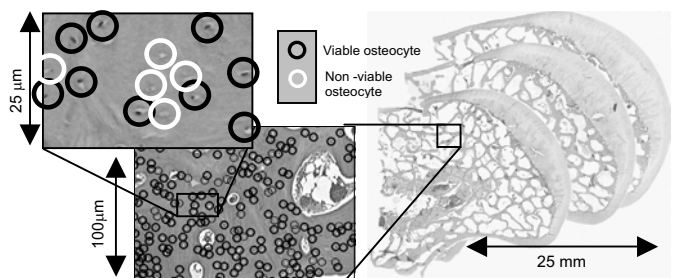


Figure 3. Stacked sections of femoral head with zoomed-in insets of an individual subimage and further zoom in of individual osteocytes.

REFERENCES

1. Reed KL, et al. *Computer Methods in Biomechanics and Biomedical Engineering* 7(1) 25-32, 2004.

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