AN ALGORITHM TO QUANTIFY SEGMENTAL LESIONS IN NECROTIC FEMORAL HEADS

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INTRODUCTION Approximately 25,000 new cases of femoral head osteonecrosis (ON) present each year in the U.S. To help clarify the pathology, a cryo-insult technique has recently been developed for creating segmental lesions1 in the emu, a large biped in which osteonecrosis progresses to femoral head collapse2. Osteonecrosis is characterized by the presence of dead osteocytes in histological sections of the affected bone. Quantification of lesion morphology is important in assessing creation of reproducible segmental lesions. An automated osteocyte identification and quantification algorithm is here reported to aid in that process.

METHODS Viable osteocytes can readily be identified in hematoxylin and eosin-stained slides by their nuclei, which appear as dark purple spots within a bright local field. Dead osteocytes present as empty lacunae – a small light-colored hole with no nucleus present – in the trabeculae (Figure 1).

An automatic detection algorithm to quantify the location of both live and dead osteocytes was written to take advantage of these morphological features. The algorithm (Figure 2), written in the PV-Wave programming language (VNI, Houston, TX), reads in eight-bit grayscale images and scans them for features signaturing osteocytes or empty lacunae. The flagged regions are then tested for aspect ratio, size, and contrast relative to the average background color. If a region is identified as an osteocyte or an empty lacuna, its centroid is computed and its status is determined by the presence/absence of a dark spot. The algorithm’s output is a list of osteocyte-center locations (pairs of x and y coordinates), and a binary value that indicates the osteocyte’s status (1=alive, 0=dead).

The algorithm also provides the user with visual feedback in the form of light and dark circles around the locations identified as live and dead respectively (Figure 3). Thus, the user can verify that the algorithm has correctly identified all points, and can manually correct any errors.

For whole-slide processing, the entire digitized slide is divided into approximately three hundred 472x472 pixel subsections, indexed according to their location on the original (complete) slide. Each subsection is analyzed using the algorithm, and the resulting data are recompiled to display osteocyte viability contour lines for the entire slide. Figure 4 shows a completed contour map for a coronal midsection of a femoral head in which osteonecrosis was cryogenically induced using a cryo-insult probe positioned subjacent to the subchondral plate, superimposed on the corresponding (pal render) histological section.

PERFORMANCE AND VALIDATION To validate the algorithm’s performance for emu cryo-insult osteonecrosis, five representative subsections (472x472 pixels each) were selected for analysis. These subsections were subjectively chosen such that the quality of histological preparation and the fractional osteocyte viability were representative of those typically seen in H&E slides of emu necrotic femoral heads. Six readers, all with formal training in histology, were given a brief instructional tutorial for manual (cursor) identification of empty versus osteocyte-filled lacunae. Each of them independently cursor-clicked on what they felt were the osteocyte-filled versus empty lacunae locations in each subsection. The manual assessments produced a total of 30 lacunae-location data files (6 readers x 5 subsections), to which the algorithm’s performance could be compared (Figure 5).

A one-way ANOVA showed that none of the human readers, nor the algorithm, produced estimates of fractional viability that were significantly different from each other (p=0.90). For none of the five subsections was the algorithm’s percentage of osteocyte-filled lacunae found to differ by more than one standard deviation from the human readers’ means. In general, the algorithm tended to predict a slightly higher percentage of viable osteocytes than did most human readers, but in no case did the algorithm’s result lie at the extreme high or low end.

DISCUSSION Until now, assessing the shape and size of segmental osteonecrotic lesions has been entirely qualitative. However, for the purposes of further developing the emu as a model for osteonecrosis, quantification of lesions now emerges as an important tool in assessing the extent of tissue damage.

As a protection against errors in image analysis, the algorithm relies on a knowledgeable human to verify the completed reads, and make corrections if needed to each file scanned. This strategy of keeping a human in the loop, while allowing the algorithm to perform the bulk of the tedious work, allows necrotic lesions to be (relatively) quickly and accurately mapped.

Fully automated histology analysis tools have been developed for “high volume” production settings, such as identifying abnormal pap smears. To our knowledge, however, such assessments have not previously been coupled with spatial registration of normal/abnormal regions, or to quantify the degree of abnormality.

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Figure 1 Stained bone showing live (L) and dead (D) osteocyte locations
Figure 2 Flowchart describing the basic algorithm design
Figure 3 Provisional lacunae identified for visual feedback (light circles = empty lacunae, dark circles = osteocyte-filled lacunae)
Figure 4 Complete osteocyte fractional viability map of an emu femoral head, resulting from a juxta-articularly located cryo-insult probe tip
Figure 5 Comparison of algorithm results to human reader results (dispersion bars = 1 s.d. in each direction)

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