INTRODUCTION:

The emu model of femoral head osteonecrosis (ON) has demonstrated the ability to mimic the debilitating collapse that characterizes the human disorder [1]. In human cases of ON, collapse of the femoral head is contingent upon both the lesion’s size and location with respect to the main weight-bearing tract. While MRI is the primary modality for routine clinical diagnosis, histological evaluation is the gold standard for definitive identification of osteonecrosis in many specialized research contexts [2].

To spatially map the distribution of osteonecrosis in the emu femoral head, a three-dimensional quantitative histology procedure has been developed and implemented. As with the human disorder [3], lesion size and location within the femoral head, are essential determinants of the propensity for femoral head collapse in this model [1]. However, due to the methods necessary to determine osteocyte viability, histologic assessment conventionally has been restricted to highly localized samplings, thus making it problematic to reliably identify the spatial distribution of osteonecrosis at the whole-head level. To address this difficulty, it is necessary to maintain registration of individual femoral head histology sections relative to the whole femur throughout processing, and to serially assemble those registered 2D sections into their correct 3D anatomic positions.

METHODS:

Cryogenically induced osteonecrotic lesions were created in a series of 10 emu femoral heads. One week post-operatively, the animals were sacrificed for femur harvest. Following fixation of the proximal 1/3 of each femur in formalin, two parallel fiducial holes 1.6mm in diameter and spaced 10mm apart were made using a custom-built leveling and drilling device. After drilling, the femoral head was decalcified. Two cylindrical dowels of semi-dehydrated potato threaded through the fiducial holes served to maintain hole visibility in the proximal emu femoral bone. The entire femoral head, with potato dowels in place, was then serially sectioned into 5-micron slices and stained with Weigert’s hematoxylin and eosin.

All histology sections were scanned through a 10x objective lens on a stepper-motor-driven microscope stage. Scans of each whole-head section resulted in a series of approximately 2200 sub-images, each of which was quantified for percentage of osteocyte-filled lacunae using a custom written Matlab program [4]. The corresponding sets of 2D data arrays from a given femoral head were then rotated and translated so as to re-align the centroids of the fiducial dowels. This resulted in a stacked 3D array representing the distribution of local percentages of viable osteocytes throughout the entire femoral head (Figure 2).

Bone was defined as being necrotic wherever there was less than 50% osteocyte viability. The necrotic sub-region was segmented from the overall 3D data set and scaled to actual physical dimensions using the known intra-slice spacing and scan area size. The nominally ellipsoidal necrotic sub-region was rotated so as to align its major axis with the x-direction. Moments of inertia were calculated around the x, y, and z-axes to index shape characteristics of the necrotic lesions. A second-order polynomial curve was best-fit to the surface of the necrotic lesion, allowing integration to determine the volume enclosed by the lesion. This volume was then normalized to that of the entire femoral head volume (Figure 3).

RESULTS:

The use of partially dehydrated potato dowels as fiducial markers provided sufficient rigidity to recover 3D registration between cancellous bone sections, while being compatible with standard paraffin embedding procedures and avoiding damage to the microtome blade during sectioning.

As expected from the cylindrical geometry of the cryoprobe (which provided coaxial closed circulation of liquid nitrogen), the induced lesions were nominally axisymmetric with their moments of inertia around the minor axes in lesion-based coordinate systems being very similar in all instances. The maximum radii of the lesions extended variably beyond the 4 mm diameter drill tract created for the insertion of the cryoprobe during surgery. Lesion radii ranged from a minimum of 4.1 mm, up to a maximum of 7.7 mm.

Also as expected, the volume of the induced lesions increased in accordance with the temperature reduction maintained during the cryoinsult, and in accordance with the number of freeze/thaw cycles utilized (Figure 4).

Figure 3. (Left) Necrotic lesion rotated to a lesion based coordinate system. (Right) Polynomial curve fits to lesion boundary: red=top edge; blue=mirror of bottom edge.

DISCUSSION:

Due to the different size scales involved, a disconnect exists between definitive histological assessment of osseous pathology, versus organ-level mapping of involvement distribution. Especially for situations such as ON, where the clinical consequences depend on the macroscopic pattern of disease involvement, it is important to overcome the obstacles posed by size scale discontinuity. The novel image registration techniques here reported provide a general means for bridging size scale discontinuity in cancellous bone. However, the large number of observations required at the microscale-level (here, typically 65,000 sub-region osteocyte viability percentage assessments per femoral head) mandates that the disorder in question lend itself to automated (or at least semi-automated) image analysis assessment of the local pathology.

REFERENCES:


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