INTRODUCTION:
Previous studies have examined differences in osteocyte and lacunar number between osteoporotic bone and normal bone using histomorphological techniques. Using a method called biomechanical stereology, Wang et al. found increased microcrack formation and propagation in fracture patients compared to normal subjects. Our goal was to determine the microscopic differences in osteoporotic bone that caused these differences. Fully empty osteocyte lacunae appear as voids (black spots) in the backscatter EM images. Bone samples from patients with a history of osteoporotic fracture had significantly more voids than normal control subjects. The larger void number explained the differences in microcrack number between the groups found in Wang et al.’s study.

METHODS:
This study examined the results from 163 finite element (FE) models that were created for 46 previously prepared human iliac crest biopsy specimens. One to five FE models were made for each individual. The biopsy specimens were imaged using quantitative backscatter electron microscopy (qBSE) and a 200 x 200 pixel section of the bone image was imported into a FE model of a compact tension fracture toughness specimen. The models of the notch specimens were solved for microcrack formation and propagation using a special purpose computer program.

The images of the microcracked regions were examined individually for morphological features associated with the microcracking. The data that was recorded included the number of cracks initiating at the notch surface, the number of voids near the notch, and the total number of voids visible in the image. Voids were defined as visible regions composed of more than one black pixel (Fig. 1). A void was considered “near” the notch if it was approximately one crack length away from the notch tip. Cracks were counted only if they initiated at the notch surface, not within a hole.

The previous work using this data analyzed the images using custom MATLAB codes. In the current study we used visual inspection by a single operator to identify and count the features of interest. In order to ensure objectivity, the reader (MS) was blinded to background of the samples, including age, race, and disease state (normal or osteoporotic).

Stat View Software was used to perform all ANOVA tests as well as Fisher’s protected least significant difference (PLSD) tests, paired t-tests, and correlation matrices.

RESULTS:
Overall, 21 of the 46 total specimens were from patients with a history of osteoporotic fracture and 76 of the 163 images examined came from this group (osteoporotic). Conversely, 25 of the specimens were from subjects with no history of osteoporotic fracture and 87 of the images represented this group (normal). Image data was averaged within each specimen and then compared by group.

The number of voids near the notch as well as the total number of voids in the image were different between groups (p<0.0001) with osteoporotics having a significantly greater number of voids both near the notch and overall compared to normal subjects. The 21 osteoporotic samples averaged 1.317 ± 0.716 (mean ± SD) voids near the notch while normal samples averaged 0.448± 0.574 voids near the notch for the 25 samples. When considering the entire 200 x 200 pixel image, osteoporotic samples averaged 3.594 ± 2.003 voids compared to 1.512 ± 1.734 for the normal samples (Fig. 2). Using a significance level of 0.05, Fisher’s PLSD method showed the mean difference between groups for the number of voids near the notch tip was 0.869 compared to the critical value of 0.396 (p<0.0001). Similarly, the mean difference between groups for the total number of voids was 2.082 compared to the critical value of 1.110 (p<0.0005).

The average number of voids per sample, both near the notch and total, was not correlated to the number of microcracks initiating at the notch as counted visually within this study. However, the total number of cracks per sample determined previously was correlated both to the number of voids near the notch (r² = 0.416, p<0.0005) and the number of total voids per sample (r² = 0.371, p<0.0005).

DISCUSSION:
The current study suggests that the differences in microcracking between fracture patients and normal subjects found by Wang et al. are associated with the number of micro-voids in the tissue. There appears to be some difference in how the holes near and far from the notch affect microcracking, but this is not yet fully quantified.

This study did not include an explicit quantification of osteocyte population but rather only counted visible holes. Fig. 1B suggests that the majority of the observed holes were osteocyte lacunae. This approach simplified the counting, but also ignored dark gray spots (low modulus) and single black pixels which could affect the propagation of microcracks. Additional examination of the types of holes that were present and whether they are essentially all osteocyte lacunae is needed.

The current study suggests that patients with a history of osteoporotic fractures might have a higher lacunar density than control subjects with no history of fracture. Based on the current study, we hypothesize a causal relationship between greater lacunar density and increased risk of fracture. It was reported that the osteocyte lacunar density of female vertebral tissue is significantly greater than that of male. If our hypothesis is correct, dimorphism in osteocyte density might be related to the dimorphism in fracture incidence between men and women.

REFERENCES:

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