Age-Dependent Loss of Osteocyte Dendrites in Human Trabecular Bone

Mia Nie[1], Rosa M. Guerra[2], X. Lucas Lu[2], Velia M. Fowler[2], Liyun Wang[2]
[1]Unionville High School, PA, [2]University of Delaware
Presenting author: mianie47555@gmail.com

Disclosures: the authors declare no conflict of interest

INTRODUCTION: Osteocytes form an extensive cellular network through their numerous dendrites [1]. These dendrites and their surrounding lacunar-canalicular pore system (LCS) play crucial roles in cell-cell signaling, metabolism, and mechanotransduction [2]. With aging, the number of osteocyte dendrites was found to decrease linearly in female mice, and the decrease outpaced that of canaliculi [3]. Previous studies have documented clear age-related changes of canaliculi in human cortical bone [4]. However, human osteocyte dendrite distributions and changes with age remain to be determined. The objective of this project was to quantitatively measure the number of dendrites emanating from osteocyte bodies using freshly obtained human bone samples. Our hypothesis was that osteocytes lose dendrites and reduce their connectivity with age.

METHODS: Femoral subchondral trabecular bone samples were obtained from seven female patients undergoing total knee replacement (Fig. 1A). These samples, being surgical remnants, were exempted from IRB approval and no medical history was provided. Within 3-4 hours after surgery, the samples were hydrated in sterile PBS for ~30 min, trimmed into smaller pieces (less than 1x0.5x0.5 inch), fixed in 4% PFA for two-three days, and decalcified at 4ºC for 6-8 weeks. The samples were cryo-embedded in OCT and sectioned (8-30 micron thick) using the cryofilm technique [5]. Following a similar protocol [4], osteocyte dendrites were stained using phalloidin (Invitrogen A12379, 1:250 dilution) and the nuclei were stained with Hoechst 33342/DAPI (Invitrogen H3570, 1:250 dilution). Per patient, 2-3 sections were stained and imaged using a Zeiss LSM880 confocal microscope and a 20x objective (image field of 425.10 x 425.10 microns). Multiple fields (3-41, median 19) per patient were captured from trabeculae located proximally to the osteochondral junction and further analyzed using NIH ImageJ/Fiji software (Fig. 1B). For each image, the DAPI channel enabled us to identify osteocytes in focus by excluding non-bone areas and out-of-focus cells using thresholding and size exclusion (Fig. 1B). The number of primary dendrites showing clear junctions with the cell bodies and extending more than a half of the nucleus radius was counted in ImageJ for the selected osteocytes (Fig. 1B). Based on individual osteocytes (from 79 to 280) analyzed per patient (Fig. 1A), histograms of osteocyte dendrites were obtained and averaged for the 50s, 60s, or 70s age groups, from which the cumulative percentage of cells with dendrites were calculated in Excel.

RESULTS: Phalloidin-stained dendrites showed limited cell-cell connectivity in 2D images (Fig. $1B_3$) and the number of primary dendrites varied from 0-20 (Fig. $1B_4$). The average histogram showed a relatively higher % of cells with 0-2 dendrites in 60s/70s age groups as compareed to the 50s age group (Fig. 1C). After peaking at 2 or 3 dendrites, the ratios of cells with higher # of dendrites were reduced, with the older groups decreasing more rapidly (Fig. 1C). The difference between 50s vs. 60s and 70s age group was clearly shown in the cumulative % of cells: 49% cells in the 50s group had no more than 5 dendrites, in contrast with the 65% and 70% of the cells in 60s and 70s groups, respectively (Fig. 1D).

DISCUSSION: Our results confirmed our hypothesis that osteocytes lose dendrites and reduce their cell-cell connectivity in trabecular bones from patients in their 70s as compared to those in their 50s. In our human knee samples, we found that the dendrite distribution shifted to fewer processes: there was a 15-20% increase in cells with 5 or fewer dendrites in patients of 60s and 70s years of age compared to those in their 50s. The degree of dendrite loss was consistent to the 30% decrease of canalicular number in a previous human study [4]. This is the first quantitative study to characterize osteocyte dendrite distribution and how the distribution shifts with aging. Limitations of the study included (1) these samples were not from healthy subjects but osteoarthritic patients, and (2) the sample size (2-3 patients per age group) was small despite the large # of osteocytes examined. More samples including males and additional concurrent assessment of canaliculi are needed for further study.

SIGNIFICANCE: Aging increases the risks of bone fragility and fracture, which negatively impacts quality of life. Our project fills a knowledge gap about aging at the cellular level and raises questions as to whether the changes are the results or drivers of tissue-level aging in human skeleton.

REFERENCES: [1]Bonewald, PMID 21254230; [2]Schaffler, 22552701; [3]Tiede-Lewis, 29074822; [4]Milovanovic, 23909715; [5]Dyment, 27684089.

ACKNOWLEDGEMENTS: First State Orthopedics and Dr. Michael Axe (human samples); UD Bioimaging Center and DCMR Research Core facilities supported by P20 GM103446; P20 GM139760. UD Graduate College Scholarship (to RMG).

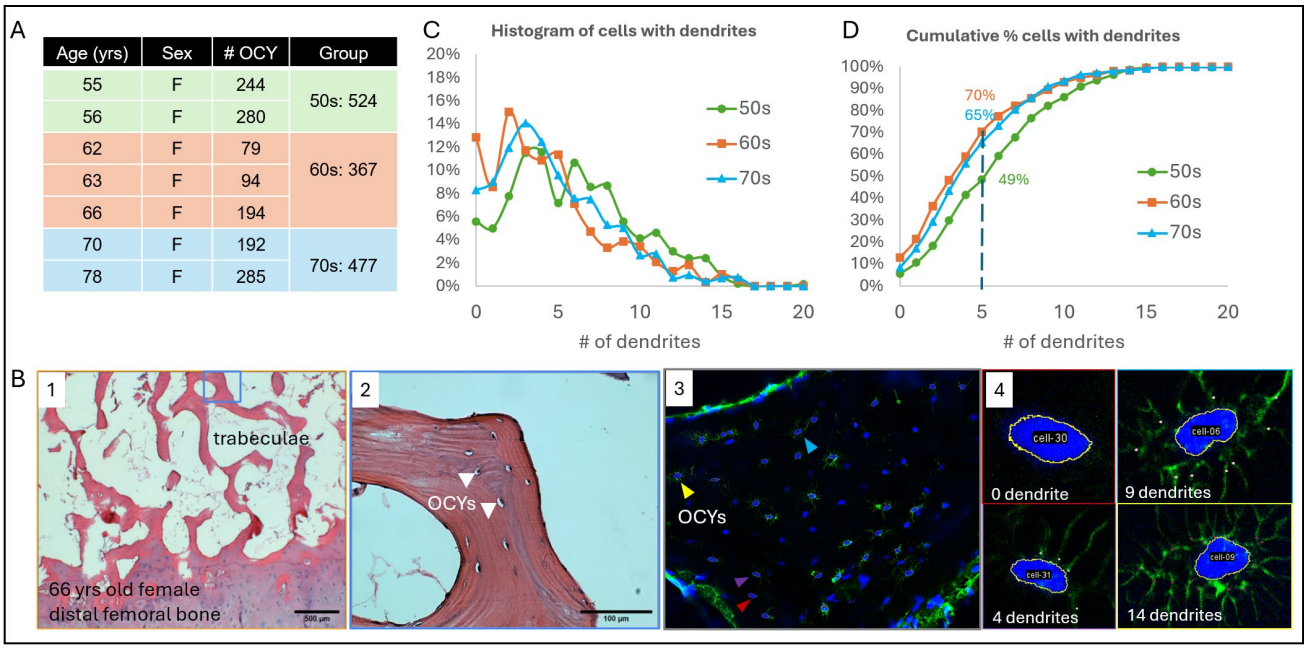

Fig. 1. *(A) Human distal femoral trabecular bone samples obtained from patients undergoing total knee replacement. (B) Representative H&E images of the bone samples (B_1) and trabecular osteocytes (OCYs, B_2). OCYs were identified using nuclear stain (blue) and dendrites were counted using phalloidin staining (B_3,B_4). (C) Histogram of the OCY dendrite numbers and (D) cumulative % of cell dendrites are shown for three age groups.*