Calciﬁcation of Articular Cartilage in the Knee of the Sprague-Dawley Rat
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INTRODUCTION:
Calcification of human articular cartilage has been found to be a common occurrence [1]. The incidence of calcification of articular cartilage in humans has been associated with aging and the progression of osteoarthritis [1-3]. Animal models with which to study the mechanisms by which pathologic calcifications of basic calcium phosphates (BCPs) form in articular cartilage have yet to be established. The aim of this work was to examine the occurrence and composition of spontaneous mineralization of the articular cartilage of the tibial plateau in the normal mature Sprague-Dawley (SD) rat using micro CT, light microscopy and scanning electron microscopy.

METHODS:
Twelve tibia plateaus from SD rats (~9 months of age) were examined. NIH guidelines for the care and use of animals were observed. Following euthanasia, each tibial plateau was excised and photographed prior to collection of a micro CT scan using a GE Explore Locus Volumetric Conebeam scanner (20.3 µm voxel size; n=12 plateaus). Specimens were formalin ﬁxed, decalciﬁed with EDTA, and parafﬁn embedded (n=10 plateaus). The posterior half of the tibial plateau was serially sectioned at 5 µm in the coronal plane. These sections were deparafﬁnized, and stained with Safranin O and Fast Green or Hematoxylin and Eosin prior to examination under light microscopy. Digital images of the medial and lateral compartments were collected and used to quantify the area of articular cartilage in each compartment that was mineralized. Five serial sections were evaluated for each plateau. The percent of articular cartilage area mineralized in each compartment was determined following manual identiﬁcation of the perimeter of each mineralized area and the total area of articular cartilage using custom software written in MATLAB (MathWorks, Natick, Massachusetts). Two specimens were ﬁxed, dehydrated and embedded in Epon-812 (Electron Microscopy Sciences). Five thick sections were prepared from each plateau and polished prior to examination using a scanning electron microscope (SEM) (Hitachi 2460N, Pleasanton, CA) using backscattered electrons (BSE) and energy dispersive spectroscopy.

RESULTS SECTION:
By gross observation, white spots were frequently visible on the articular surface of the tibial plateau (Fig. 1A). Review of the micro CT scans found mineralization of the articular cartilage (Fig. 1B) present in 12/12 of the tibial plateaus examined. These areas of mineralization coincided with regions of white spots observed grossly (Fig. 1A) and cartilage cysts observed histologically (Fig. 2). These regions of mineralization occupied 1.03 % [0-14.21 %], mean [range], and 1.47 % [0-6.83 %] of the articular cartilage area in the medial and lateral compartments respectively. Scanning electron microscopy revealed that the mineralized areas were comprised of discrete particles (Fig. 3A). Elemental analysis found these regions to contain mainly oxygen (61 atomic %), phosphorus (15.1 %) and calcium (22.6 %) (Fig. 3B). The mean calcium to phosphate ratio was 1.50±0.038 (from n=8 randomly sampled particles) which is consistent with the mineral phases present by x-ray diﬀraction. The morphology and composition of mineralizations observed in this work appear consistent with those previously reported in human articular cartilage that were identiﬁed as primarily basic calcium phosphate (a class of compounds including hydroxyapatite, tricalcium phosphate and octacalcium phosphate) [3]. The SD rat may provide an animal model with which to study the development of basic calcium phosphate mineralizations and their effect on the resulting properties and function of the articular cartilage.

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