INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by progressive erosion of articular cartilage and changes in the underlying subchondral bone plate (ScBP). The pathogenesis of OA is associated with abnormal mineralization activity resulting in new regions of calcified cartilage (CC), and increased vasculature, including the invasion of vascular canals beyond the tidemark. The vascular canals in CC may modulate the load-bearing behavior of articular cartilage by contributing to the hydraulic conductance of the ScBP. We hypothesized that increased hydraulic conductance of the ScBP is associated with the progression of OA. The objectives of this study were: (1) to determine and compare the ScBP hydraulic conductance for different grades of erosion of the osteoarthritic human femoral condyle, and (2) to evaluate the contribution of residual cartilage to the hydraulic conductance of fully eroded ScBP in OA.

METHODS: Sample Harvest: Osteochondral cores (9mm diameter) were harvested from the medial femoral condyles of tissue bank donors and discarded knee fragments from total knee replacement surgery patients with IRB approval (Fig. 1). Each core was obtained from a different donor, and adjacent osteochondral fragments were taken for histology. Tissue bank donor cores were considered normal (n=7), and OA cores were graded by visual inspection as having partial erosion (n=10) or full erosion to eburnated bone (n=11 total, n=7 used for Exp. 1 and n=4 used for Exp. 2). Experimental Design: Exp. 1 - Effect of OA erosion: Uncalculated cartilage was removed from normal, partially eroded, and fully eroded cores by 18-24h digestion with 400 U/L papain. Before perfusion testing, a low-speed bone saw was used to trim cores to a 5mm height including the entire ScBP, from the CC layer at the surface to trabecular bone on the bottom. Exp. 2 - Effect of residual cartilage in ScBP of fully eroded samples: Fully eroded cores were perfusion-tested first in the intact state at harvest and again after removal of any residual cartilage by papain digestion. Perfusion Testing: Each sample was inserted into Tygon tubing, sealed circumferentially, and tested for hydraulic conductance, as previously described. PBS was perfused through samples at constant flow rates (0.1-15 mL/min) controlled by a syringe pump, and pressure drop was measured across the sample. Samples were tested with flow towards and away from the CC surface to determine effect of flow direction, with three trials in each direction to assess repeatability. Darcy’s Law was used to estimate the hydraulic conductance constant [m/(Pa*s)], of each sample using a least squares fit of fluid velocity [m/sec] vs. pressure drop [Pa]. Histology: Osteochondral fragments taken adjacent to each core site were split into two pieces; one left intact (Fig. 2A-C) and the other papain-digested along with the core (Fig. 2D-F). Fragments were fixed in 4% paraformaldehyde and decalcified in 20% EDTA. For 2-D histology, samples were snap-frozen in OCT, cryosectioned at 5μm thickness and stained with Toluidine blue. For 3-D histology, samples were fluorescently stained with Eosin-Y, embedded in resin, and imaged at (2.24 μm)3 voxel resolution. Statistics: Conduction values were log10 transformed to improve homoscedasticity. Data are presented as mean ± SEM. A repeated-measures ANOVA was used with test trial as the repeated effect to determine treatment effects of osteoarthritic erosion or papain digestion. Post-hoc comparisons were performed with Tukey. Linear regression was used to assess the effect of flow direction.

RESULTS: Exp. 1: Hydraulic conductance of the ScBP increased with severity of OA erosion (Fig. 3). Conductance estimates were reproducible within samples (not significantly different) for different trials. Conductance estimates with flow going towards and away from the CC surface were also strongly correlated (r²=0.95). Exp. 2: Papain digestion increased hydraulic conductance in some fully eroded OA samples but had no effect in others (Fig. 4). Histology: 2-D and 3-D histology results were qualitatively consistent. Papain digestion removed uncalcified cartilage but preserved the CC structure (Fig. 2). OA samples exhibited large variations in CC thickness (Fig. 2B,C,E,F), with fully eroded samples having a thickened cortical endplate (Fig. 2C,F).

DISCUSSION: Hydraulic conductance properties across the bone-cartilage interface are altered in osteoarthritic disease states. Such an alteration may cause differences in deep-zone chondrocyte and extracellular matrix metabolism. Residual cartilage may play a variable role in the hydraulic conductance of fully eroded OA ScBP although the location of the solubilized tissue, at the surface or within the ScBP, remains to be established. Delination of the fluid transport characteristics of the bone-cartilage interface, and also the compositional and structural determinants of such characteristics, will help to understand the interaction between the microenvironments of subchondral bone and deep-zone cartilage.


ACKNOWLEDGEMENTS: HHMI, MTF, NFL, NIH and NSF (JH)