ISB 2013 BRAZIL
XXIV CONGRESS OF THE INTERNATIONAL SOCIETY OF BIOMECHANICS
XV BRAZILIAN CONGRESS OF BIOMECHANICS

NANOINDENTATION OF THE HUMAN FEMORAL HEAD

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SUMMARY
The mechanical aetiology and progression of osteoarthritis in articulating joints is not fully understood. We have characterised the variation in the nanoindentation stiffness of articular cartilage in non-OA and OA human femoral heads. The superior region of the head is stiffest, and where most loss of cartilage is seen. The region surrounding the fovea capitis is most compliant and may show signs of early mechanical breakdown, which we hypothesise is associated with shear stress.

INTRODUCTION
Articular cartilage (AC) is a remarkable tissue in which a complex composition and hierarchical structure provides an optimized function in load support and joint lubrication. OA may be considered as the degeneration and consequent loss of AC. Since AC has no blood supply, it has a poor healing capability when physically damaged [1], however, daily contact stresses play an important role in maintaining the hip joint and can help to strengthen the AC [2]. OA alters the structure and composition of AC which leads to an altered mechanical function and pain.

The mechanical properties of AC are regional and depth dependant [3], however a direct comparison of local mechanical properties across degenerated human femoral head cartilage has not been thoroughly investigated. Nanoindentation is a nondestructive technique often used for the mechanical analysis of healthy AC [4]. Nanoindentation has the capability to measure mechanical properties at scales at which the curvature of the femoral head is irrelevant. With this technique, AC stiffness determined from the unloading curve is simple to calculate and does not involve many assumptions unlike other mechanical properties. This paper identifies and quantifies areas of degeneration on surgically explanted femoral heads following total hip replacement (THR). Comparison with age-matched cadaveric non-OA femoral heads yields insight into areas in which degeneration may initiate.

METHODS
Following ethical permission, twelve human femoral heads were harvested following THR surgeries and collected via the NHSGGC Bio-repository. In addition, six fresh-frozen hemi-pelvis to toe cadaveric legs were obtained from Anatomy Gifts Registry (Hanover, MD, USA). These latter specimens had no medical history of OA and had been thawed once for knee arthroplasty surgical training. The six cadaveric femoral heads were removed following a typical surgical protocol. Specimens were stored frozen at -18 °C prior to sample preparation. All femoral heads were submersed in PBS during sample preparation. Twenty two osteochondral plugs were removed from specific areas on each femoral head, Figure 1abc. The plugs included the full cartilage depth and approximately 2mm of subchondral bone. The osteochondral plugs were washed over night in PBS to remove surface synovial fluid. After washing, the subchondral bone was secured in a petri dish using Plaster of Paris with the full thickness of cartilage protruding. The petri dish was then filled with PBS up to the cartilage surface.

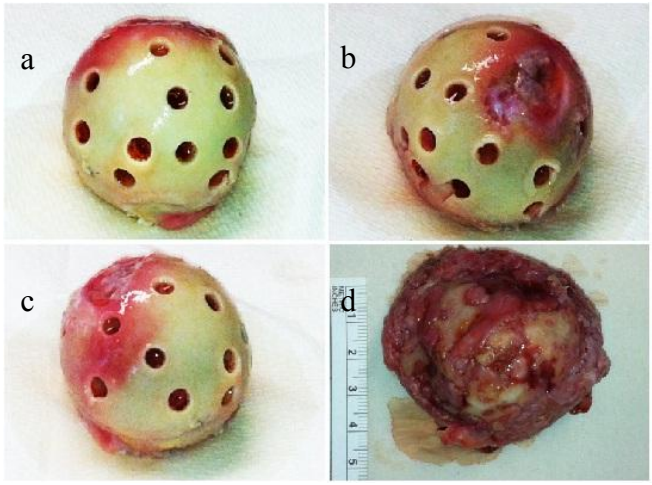

Figure 1: a,b.c: A single cadaveric femoral head with 22 osteocondral plugs removed. d. is an example of a severely degenerated femoral head explanted from THR surgery.

A lower-force MFP Nanoindenter (Asylum Research, Santa Barbara, CA), fitted with a diamond Berkovich tip, indented the cartilage surface to a depth of 5μm. Manual surface detection was used to ensure a precise measurement of indentation depth as considerable errors were observed when automatically triggering the compliant surface. 16 indents were performed at different locations in a 50 μm x 50 μm area in the centre of each sample. A triangular indentation function was applied with displacement and retraction rates of ±5μm/s.

Samples were qualitatively grouped into 3 grades: samples with missing AC were denoted grade '2', whilst mechanically 'normal' AC was classified '0'. Frequently, a surface response was elicited whereby the surface stayed attached to tip during full retraction resulting in permanent deformation. In this case a grade of 1 was recorded for the sample. Grade 1 force-retraction curves were unreliable for quantitative analysis.

For grade '0' samples, force displacement curves were analysed and the stiffness (*S*) was calculated from the force-displacement gradient at the start of tip retraction. Stiffness data were analysed using a repeated measures ANOVA evaluating the variance in *S* due to cadaveric or surgically explanted femoral head and due to grouped anatomical location of sample. Statistical significance was taken at $p<0.05$. Data are presented as mean ± SD.

RESULTS AND DISCUSSION

The surgically explanted samples had significantly more class 1 and class 2 samples than the non-OA group (Table 1, χ^2 test, $p < 0.001$) The worst affected area of OA in the surgically explanted samples was in the superior-posterior area of the femoral head (Figure 2). The regions of absent AC match well with those predicted to have higher contact stress [5].

	Class 0	Class 1	Class 2
Surgical (n=264)	**33%**	**42%**	**25%**
Cadaveric (n=132)	**86%**	**14%**	**0%**

Table 1: % number of samples in each class

Since many samples (42%) from the surgically explanted heads were classed as grade 1, this arguably suggests that grade '1' mechanical behaviour was indicative of AC degeneration. The cadaveric femoral heads showed no (0%) indication of end stage OA, however some samples close to the fovea capitis or in the anterior area at the edge of the femoral head were grade '1' and thus potentially demonstrated symptoms of degeneration.

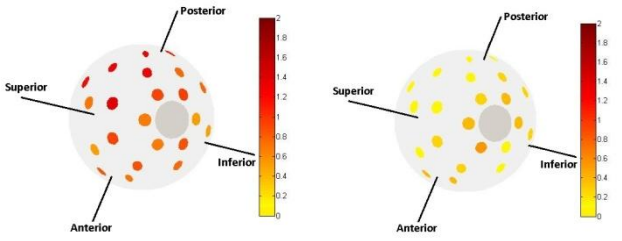

Figure 2: Qualitative analysis of explants and cadveric femoral heads. Dark Red (2) is equal to bone and Yellow (0) is equal to cartilage.

The cadaveric tissue has an average stiffness (181 N/m) similar to prior studies [4] and significantly greater ($p=0.009$) than that of the surgically explanted femoral head AC (138 N/m). Stiffness also significantly varied between anatomical location ($p = 0.005$), with subsequent post hoc tests highlighting a significant difference between superior and medial locations (Figure 3). Matching the grade 1 distribution, the most compliant AC region was found medially around the fovea capitis in both the surgical and cadaveric samples. The stiffest region for both groups was in the superior zone, in agreement with FE modelling [5].

We hypothesise that the location of the grade 1 indentation behaviour about the fovea capitis may be the result of shear stress damage on the AC surface potentially removing the superficial layer of cartilage. The abundance of proteoglycans in the middle zone may attach to the indenter creating the observed mechanical behaviour. The loss of superficial layer may also occur during excessive loading normal to the surface, e.g. in the superior region of the femoral head, as a precursor to total cartilage loss. Histology and microscopy is underway to ascertain compositional and microstructural differences between the samples.

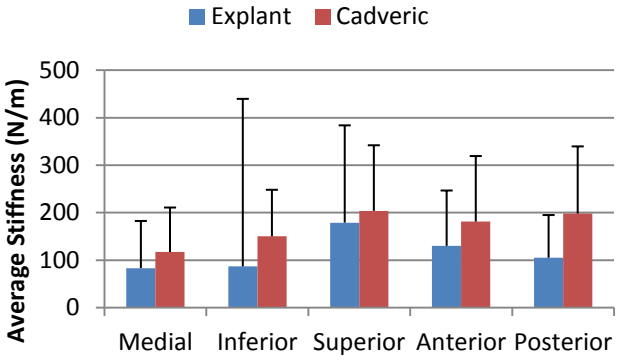

Figure 3: Average grouped stiffness's for surgical and cadaveric femoral head AC (mean + SD).

CONCLUSIONS

The most affected area of AC on the femoral head by OA is the superior posterior region, which is also the stiffest region when healthy. A potential biomechanical biomarker of OA has been found, in which the surface attaches to a Berkovich indenter during indentation, and a hypothesis presented for its existence.

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