Novel Biomechanical Indentation Test Demonstrates Joint Surface Weakening in Mice Lacking Fibroblast Growth Factor Receptor 3 (FGFR3)

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INTRODUCTION

Recent advances in murine molecular genetics has enabled the production of mice with targeted disruption of genes that regulate cartilage and bone metabolism. These mice represent excellent models to study the etiology and progression of complex diseases, including arthritis. Mice lacking FGFR3-/- exhibit defects in cartilage and bone metabolism by 4 months of age (**Figure 5 to 7**).

Figure 1: *Indentation Montage*

METHOD

FGFR3-/- mice were used as a prototype to develop a biomechanical indentation method to quantify changes in the surface load bearing properties of articular cartilage overlying subchondral bone. Right and left humeri were harvested from FGFR3+/+ and FGFR3-/- mice and the distal metaphysis fixed with cement in a small plastic container before dissection of the joint capsule to expose the articular surface (**Figure 2**). The immobilized humerus was then immersed in PBS for 30 minutes for equilibrium. Controlled compression was applied by an actuator with 0.1 µm displacement precision moving a flat tip indentor of 60 µm diameter positioned perpendicular to the articular surface (**Figure 3**). Ramp compressions at 5 µm/s was applied at two different positions on each humeral head and the force response captured by a load cell with 7 mg precision.

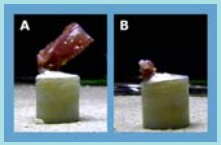

Figure 2: *(A) distal metaphysis of the humeral joint fixed in cement and (B) after joint capsule removed.*

BIOMECHANICAL CHARACTERISATION

The biomechanical properties of FGFR3 +/+ and FGFR3 -/- cartilage were compared using six measurements from the captured force/response curve as variables for statistical analysis. Three slopes (g/um) were measured between 2.5–5.5 um, 5.5-8.5 um and 8.5-11.5 um and three forces (g) measured at 4, 7 and 10 um of compressive displacement (**Figure 4**).

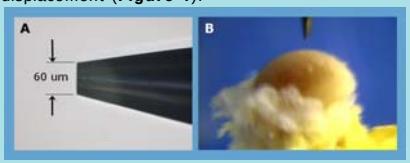

Figure 3: *(A) Dimension of the indentor tip. (B) Indentor positioned perpendicular to the cartilage surface before starting the routine.*

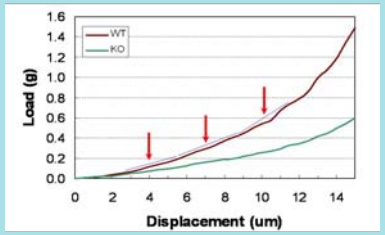

Figure 4: *Typical force response curves from indentation of WT and KO cartilage. The three forces (↓) and the three slopes (⌐) are shown for the WT.*

PATHOLOGY

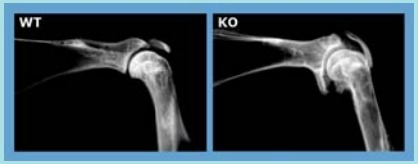

Figure 5: *Faxitron x-ray of humeral head showing changes in morphology and reduced joint gap in FGFR3-/- (KO) mice compared with wild type (WT) littermate control at 4 month of age.*

Figure 6: *Coronal sections and 3D reconstruction of humeral head obtained with micro-computed tomography (microCT). KO shows narrowing of joint gap, indicating thinner cartilage, and defects in subchondral bone compared with WT control.*

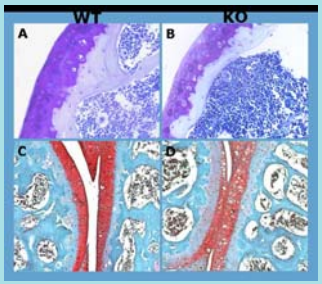

Figure 7: *Histological analysis of humeral head stained with Von Kossa/toluidine blue **(A-B)** and Safranin O **(C-D)** showing a decrease in proteoglycan in the KO compared with WT mice.*

RESULTS

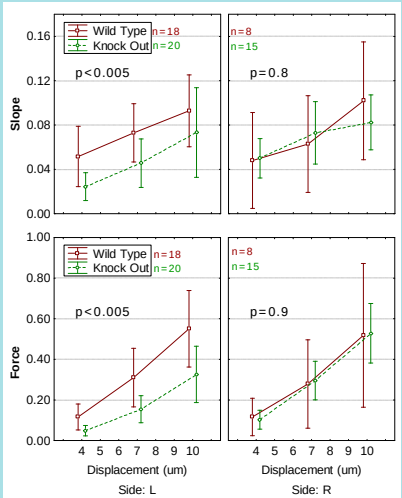

Figure 6: *A significant reduction in joint surface indentation stiffness (both the slope and force) for WT compared to KO was found for the left humerus but not the right. MANOVA with WT/KO as categorical predictor and displacement as continuous predictor was used.*

CONCLUSION

Novel technology has been developed to test the biomechanical properties of mouse articular cartilage. It was determined that the load bearing surface of the left humeral head of FGFR3-/- mice was significantly less stiff ($p<0.005$) than that of wild type littermates when measured at 5 µm/s velocity. The lack of difference seen in the right joint is intriguing Given the progressive degenerative changes that occur in the articular joints of FGFR3-/- mice, starting at 4 months of age, it is predicted that the biomechanical indices of joint degeneration will be more apparent in 6-8 month old mice. This novel method appears to be capable of quantitative assessment of joint surface biomechanics in genetically modified mice.