Introduction: An early indicator of osteoarthritis (OA) is a decrease in proteoglycan (PG) content, which in turn decreases the fixed charge density (FCD) of the tissue. Because cartilage is triphasic, an accurate assessment of the FCD is possible by measuring the intrinsic (matrix only) and apparent (matrix+osmotic pressure) properties of the tissue via indentation testing (1). Two analytical methods for indentation testing have recently been proposed: the “dual-stage” method to calculate FCD from the mechanical properties of the tissue when tested in isotonic (apparent properties) and hypertonic solutions (intrinsic properties); and the “single-stage” method to predict FCD as for the dual-stage method assuming an intrinsic Poisson’s ratio of 0.05 (1). The “single-stage” method is preferred since only one indentation test is required to ascertain the apparent properties. Our objective was to compare the accuracy and reliability of these two methods for measuring FCD in normal and degenerated osteochondral specimens.

Materials and Methods: Pairs of osteochondral specimens from the right and left medial tibial plateaus of seven adult goats were obtained. One specimen from each pair was randomly selected to undergo chemical degradation to remove PG while the contralateral specimen served as a control. PGs were reduced by incubating the specimen in 2.5 units of chondroitinase ABC at 37°C for 24 hours. Indentation creep tests were performed on a miniature material test system (Bose 3200). Compressive loads (3.5 g tare load for 15 minutes followed by a 10 g test load for 150 minutes) were applied through a 1.5 mm diameter flat porous tip (2, 3). The specimens were tested while bathed in hypertonic solution (2.0M PBS) so that the intrinsic material properties were established (1). The indentation tests were then repeated with the specimen in isotonic solution (0.15M PBS) following a 2 hour recovery period. A biochemical analysis using the 1,9-dimethylmethylene blue (DMMB) assay was conducted to directly measure PG content and hence FCD of cartilage cores taken from the indentation sites (1). The apparent and intrinsic properties were calculated using the biphasic analysis software developed by Mow et al (2). For the dual-stage test, the material properties were directly measured from the indentation tests in 2M (intrinsic) and 0.15M (apparent) solutions. For the single-stage test, the material properties from the 0.15M solution (apparent) were directly measured. Equivalence between the shear modulus under both test conditions was utilized, and the intrinsic Poisson’s ratio was assumed to be 0.05. The FCD was then established by calculating the change in the lame constant, \( \lambda \), between the apparent and intrinsic conditions as previously described (1). ANOVA was used to compare the FCD values between techniques and cartilage status. Regression analyses were performed to determine which method provided the most reliable FCD predictions using DMMB as the gold standard.

Results: There were no significant differences between the single-stage, dual-stage and DMMB methods for establishing the FCD (p=0.61). The mean (±SD) FCD values measured using the dual-stage method were the closest (control: 0.129±0.039; degenerated: 0.046±0.029) to the DMMB results (control: 0.125±0.034; degenerated: 0.057±0.024) when compared to those of the single-stage method (control: 0.147±0.035; degenerated: 0.063±0.026). However, the FCD values for the degenerated specimens were significantly lower than the control specimens (p<0.01). The single-stage method was the most reliable (Fig. 1; r²=0.81) when compared to the dual-stage method (Fig. 2; r²=0.79).

Discussion: An evaluation of the two indentation approaches to predict the FCD revealed that the single-stage method provided an accurate and reliable FCD prediction when compared to the DMMB measurement of PG content (assumed to be the gold standard for this study). Both methods demonstrated that the loss of PG in degenerated cartilage resulted in lower FCD values. These data highlight the fact that a reduction in PG results in a decreased ability of the degenerated cartilage to resist compression. The decrease in negative charge decreased the resistance to fluid flow in cartilage and thus lowered its resistance to compression. Since both methods provided relatively accurate and reliable FCD estimates in normal and degenerated tissues, the more efficient single-stage method was determined to be the ideal technique for predicting FCD and hence PG content of the tissue.