INTRODUCTION:
Anterior cruciate ligament (ACL) knee injuries are prevalent and are a key contributing factor to the etiology of osteoarthritis (OA) [1]. The initial changes to periarticular bone and secondary supporting structures in a post traumatic anterior cruciate ligament transected (ACLX) joint occur due to an imbalance in bone remodeling. Periarticular bone changes can be detected early in disease progression [2] in humans [3] and animal models [4,5]. Changes to the secondary supporting structures (e.g. medial collateral ligament–MCL) also occur early in disease progression in experimental OA [5]. The changes include a loss of joint stability due to an increase in MCL bone complex laxity, which correlates to an increase in MCL insertion volume [6].

Anti-catabolic bisphosphonate (BP) therapy (risedronate) inhibits bone remodeling [7]. Through BP therapy, MCL complex laxity [8] was conserved in experimental OA. Whether MCL complex laxity was conserved solely due to a reduced MCL insertion volume [6], or because periarticular bone architecture and mechanical integrity were also conserved is not clear.

Therefore, the purpose of this study was to determine the effect of BP therapy on the architecture and mechanical stiffness of periarticular bone through a morphological and finite element analysis using micro-computed tomography.

METHODS:
Skeletally mature, female New Zealand white rabbits were randomly assigned to four groups (N=8/group). All animals were sourced from the same supplier (Riemens Fur Ranches, St. Agathe, ON). The anterior cruciate ligament was transected and the animal was anesthetized. A 7.6 mm (200 slices) sample of the medial condyle containing the MCL and the distal 5 cm of the femur was dissected. Samples were placed into a polystyrene vial in 4% paraformaldehyde and capped to reduce dehydration. The samples were dosed with BP (RISE) (risedronate, 0.01 mg/kg s.c. daily for 6 wk), and the second group was untreated (ACLX). The third group comprised unoperated normal controls (NCON), and the fourth group was sham operated controls (SHAM). After 6 wk, all animals were sacrificed by barbiturate overdose, and the distal 5 cm of the femur was dissected. Samples were placed into a polystyrene vial in 4% paraformaldehyde and capped to reduce dehydration.

The distal 28.2 mm of the rabbit femur was scanned using micro computed tomography (μCT, vivaCT 40, Scanco Medical, Switzerland) using a standard protocol (55 kVp, 109 μA, 300 ms integration time, frame averaging of 1, 38 μm isotropic voxel size).

A 7.6 mm (200 slices) sample was analyzed to determine the following morphological parameters: bone volume ratio (BV/TV), bone surface to bone volume ratio (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (Conn.D), structure model index (SMI), degree of anisotropy (DA), and cortical thickness (Ct.Th).

The apparent and tissue level mechanical stiffness properties of the trabecular bone were determined through finite element (FE) analysis. A 3.8 mm (100 slices) sample of the medial condyle containing the MCL insertion was extracted from the region of interest analyzed during the morphological analysis. Finite element meshes with hexahedron elements were automatically generated using the voxel conversion approach. The boundary conditions were uniaxial displacement to 1% strain. The FE tissue material properties were assigned isotropic homogenous linear elastic modulus (10 GPa, Poisson’s ratio 0.3).

An ANOVA (SPSS, Chicago, IL, USA) was completed for each morphological and finite element measurement to determine the presence of statistically significant differences between groups. If a significant (p<0.05) difference was found between groups using the ANOVA, contrasts (t-test) were performed.

RESULTS:
Trabecular bone architectural differences between the four groups were visible with qualitative analysis and corroborated with the morphological and finite element analysis results. The NCON and the ACLX groups differed significantly for BV/TV (p=0.02). The RISE group was not significantly different from the NCON group for BV/TV (p=0.64), indicating that treatment conserved this morphological variable. A non-significant trend of conservation using BP therapy was consistent for the other morphological measurements.

The finite element analysis did not yield statistically significant results, however, at the apparent level; the mean values for reaction force (ACLX 622 ± 83 N, NCON 701 ± 103 N, RISE 715 ± 109 N) indicated BP therapy may have produced a trend toward conservation of bone stiffness. At the tissue level, strain energy density (ACLX 0.096 ± 0.0062, NCON 0.0951 ± 0.0080, RISE 0.0966 ± 0.0063) and average Mises stress (ACLX 24.5 ± 1.0 MPa, NCON 25.3 ± 1.3 MPa, RISE 25.4 ± 1.2 MPa) results also indicated a non-significant trend towards a conservation of bone tissue stiffness through BP therapy. No significant differences were found between the unoperated normal controls and the sham operated controls for the morphological or finite element measurements, but a trend towards bone loss and a decrease in mechanical apparent level and tissue level stiffness was observed.

DISCUSSION:
The purpose of this study was to determine the effect of BP therapy on the architecture and mechanical properties of periarticular bone through a morphological and finite element analysis. The results demonstrated that BP therapy conserved bone, and supported the hypothesis that conservation of bone mechanical stiffness may contribute to normal MCL complex laxity found in BP treated experimental OA.

Sample size was a limitation of this study, as biological variance played a significant role in the variability of some measurements. That variability may have been affected by visible osteoarthritic changes (i.e., meniscal tears and of osteophyte formation) appearing in some ACLX rabbits and not in others, indicating a variation in disease progression.

The results indicated that BP therapy reduced MCL complex laxity not only through conservation of bone mineral at ligament insertion sites, but also likely through a conservation of underlying trabecular bone stiffness. Conservation of trabecular bone stiffness may have had a secondary effect on internal loading conditions as trabecular bone likely provided support to the subchondral plate which is a primary load bearing structure. Therefore, the effects of BP therapy on the post traumatic ACLX joint may have been two-fold: the initial bone loss resulting from limb disuse due to injury may have been reduced, and long term bone loss that occurred from altered internal loading conditions may have been reduced due to a decrease in joint laxity and a conservation of bone mechanical integrity. The current results provided further evidence for the potential interventional role of BP therapy in the prevention of long-term OA development.

REFERENCES:

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