Monitoring Load with Segmental Bone Replacements during Repetitive Impact Loading

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Statement of Purpose: The loads and loading rates responsible for the induction of osteoarthritis (OA) are largely unknown. Monitoring loads and loading rates during the induction of osteoarthritis will provide crucial information regarding the mechanical factors responsible for osteoarthritis. Additionally, this can be analyzed in conjunction with the biochemical and genetic factors hypothesized to play a role in OA to determine the relative importance of each factor in the induction of OA. Repetitive Impact Loading (RIL) has been shown to induce OA in rabbit and guinea pig animal models and recent preliminary work has shown this is also possible in the mouse model. The purpose of this study was to monitor loading during repetitive impact loading using a novel segmental bone replacement sensor. Bone load sensors offer the advantage of being calibrated prior to implantation so that loads can be calculated from strain without having to calculate the moment of inertia or the stiffness of the bone.

Methods: The novel bone load sensors were made using a custom injection mold that was designed using dimensions from micro-CT data of a mouse femur. The segments for the load sensors were formed from polybutylene terephthalate (PBT) and were 4mm long with 2mm intra-medullary stems on both ends. A 120-ohm strain gauge was attached to the implant using Masterbond epoxy (Masterbond Inc, Hackensack, NJ) and calibrated on a Material Testing System (MTS Corp, Eden Prairie, MN) so that strain could be converted to load. Load sensors were implanted into the left hind limb of mouse cadavers. Measurements from load sensors and strain gauges directly attached to the femur were compared during the RIL loading procedure. The RIL procedure consisted of holding the left hind limb of the mouse in full extension and loading the calcaneus at 5 Hz. The *in vivo* RIL procedure was performed 5 days a week for 15 minutes a day and at 5 Hz for either 2 or 4 weeks to mark an early and later time point. Following the procedure the mice were sacrificed, paraffin embedded and stained with H&E and Safranin-O. Loaded limbs were compared to unloaded limbs and examined for markers of OA including surface fibrillations, cell densities and intensity of Safranin-O staining.

Results: *In vivo* loading following the RIL procedure resulted in noticeable surface fibrillations of the femoral articular cartilage particularly at the 4 week time point (Figure 1). Additionally, one mouse was showing clinical signs of arthritis (limping and decreased range of motion) after 1 week of loading. It was also noted that cell density (cell #/mm^2) was decreased 41% in the tibial cartilage and 52% in the femoral articular cartilage. Safranin-O staining demonstrated a decrease in staining intensity in the loaded versus the unloaded limb (Figure 1). Load monitoring of segmental bone replacements that were implanted in mouse cadavers and loaded according to the same RIL loading procedure demonstrated an average coefficient of variance (Standard Deviation/Mean) of 0.05±0.03 compared to 0.07±0.04 for a strain gauge directly attached to the femur (Figure 2). This difference was not statistically significant (p=0.46). Additionally, it was noted that the segmental bone replacement measured 130% of the load that was applied to the calcaneus.

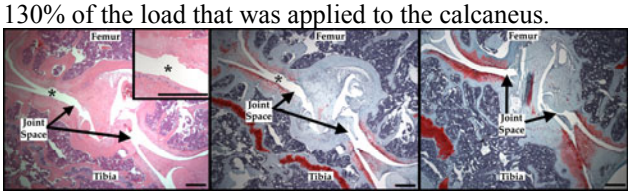

Figure 1. (Left) H&E stain demonstrating surface fibrillation on the femoral condyle *. Inset is a high magnification image. (Middle) illustrates decreased intensity of Safranin-O staining in the loaded limb compared to the unloaded limb (Right). All scale bars are 250 micrometers.

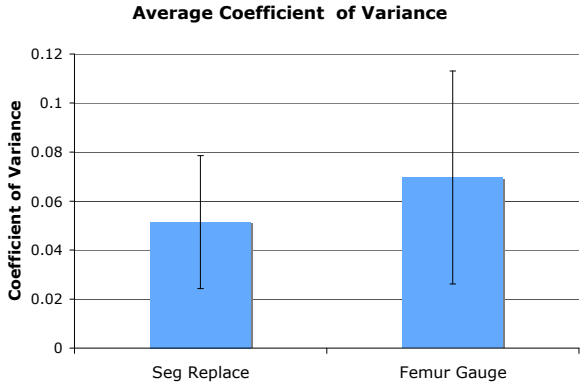

Figure 2: Bar graph comparing the coefficient of variance for the segmental bone replacement load sensor to a gauge attached directly to the femur. This difference was not significant (p=0.46).

Conclusions: Histological evaluation of the knee joint following *in vivo* RIL demonstrates the ability of RIL to induce OA. This offers a mouse model of OA, which can be used to study the effects of loads and loading rates on the induction of OA. *In situ* testing of the segmental load replacement demonstrates that it will provide equally precise data monitoring when compared to a strain gauge directly bonded to a femur. Additionally, the segmental load sensor can be calibrated prior to implantation, which will allow conversion of strain to load. While strain gauges directly attached to the femur can be used to calculate load, this requires CT scanning to calculate the moment of inertia and stiffness. Over time as bone grows around and attaches the strain gauge to the bone both the moment of inertia and stiffness will change, and this will necessitate serial *in vivo* CT scans of the mouse femora in order to calculate load over the time course of the study. Future research will be directed towards *in vivo* monitoring of segmental bone load sensors during RIL and correlating load to the pathological changes in the cartilage.