Introduction: Osteoarthritis (OA) is a degenerative joint disease characterized by the loss of articular cartilage, remodeling of subchondral bone, osteophyte formation, and limited synovial inflammation. Studies of early primary OA in humans are difficult because the course of the degenerative process is slow. However, OA-like conditions occur naturally in animals such as horses, pigs, dogs, and guinea pigs. Spontaneous degeneration of the knee joint cartilage in male Dunkin-Hartley (DH) guinea pigs has been well characterized microscopically and serologically [1,2,3], and this model is widely used to study the pathogenesis of cartilage degeneration and to evaluate potential anti-arithmetic drugs for clinical use. In this study we used stereological cartilage morphometric analysis and micro-CT to compare the three-dimensional changes of cartilage and subchondral bone in the DH guinea pigs versus GOHI guinea pigs that are less prone to developing spontaneous OA. In addition, we investigated the effect of intrarticular hyaluronan injection on cartilage and biomarker changes in the DH model of OA.

Materials and Methods:

Animals & treatment: Three groups containing six animals each were studied: 1. DH arthritic guinea pigs; 2. DH arthritic guinea pigs treated with hyaluronan; 3. GOHI strain guinea pigs. The guinea pigs were 6 months old at the onset of investigation. The treatment group received 3 intraarticular bilateral injections of sodium hyaluronate (0.1ml, 10mg/ml, Pfizer, MW≥3million) at the ages of 6, 6.5, and 7 months.

Biomarker: Biweekly urine samples were collected from the 6-9 month old from control DH and GOHI guinea pigs, and four urine samples were collected from the 7-9 month old HA-treated DH guinea pigs. Urinary Type-II collagen breakdown products Col2Ctcs were evaluated by ELISA using the urine samples.

Stereological study of the cartilage: Animals were sacrificed at 11 months old. The proximal tibial plateaus were dissected free of soft tissues and processed by dehydration and plastic embedding. The proximal tibias were serially sectioned, polished and surface stained. The percent lesion volume and mean defect depth were evaluated systematically using unbiased stereological methods [3] (Figure 1). The thickness of the hyaline articular cartilage (AC) layer, the calcified cartilage (CC) and subchondral bone plate (SCP) were measured from the central section of each medial tibial plateau.

Micro-CT assessment of the subchondral bone: Dissected femurs were scanned with micro-CT to quantify three-dimensional microarchitecture of subchondral plate, cancellous bone and the osteophyte formation. To explore site-specific changes in microstructure, four regions of interest in the femoral epiphyseal cancellous bone were analyzed, which include medial and lateral aspects of the anterior and posterior sites.

Results: Cartilage morphometric analysis revealed more severe lesions in DH other than GOHI guinea pigs, which are similar to those seen in human OA. In general, tibia plateau cartilage was thicker on the medial than on the lateral side. The small, superficial lesions were usually associated with a thickening of the cartilage layer and chondrocyte clustering. Compared to the GOHI strain of guinea pig, the DH one exhibited a significantly larger lesion volume (3.8% vs. 1.5%) and a thicker AC (0.042 vs. 0.035mm), but a thinner CC (0.008 vs. 0.01 mm) and SCP (0.035 vs. 0.039 mm). The micro-CT analysis revealed the DH strain has a significantly lower bone mass than the GOHI guinea pigs including a) a lower bone mineral density, b) a smaller bone volume fraction and bone surface area and c) an increased spacing between the subchondral trabeculae. The DH strain was also characterized by larger osteophyte volume. The aforementioned changes were most severe on the medial side of the joint, particularly in the anterior region.

HA treatment group did not show significant structural difference than the non-treated DH guinea pigs in all analyzed cartilage morphological parameters. Normalized urinary Col2Ctcs was increased in the DH guinea pigs compared to GOHI strain during the disease progression (most DH guinea pigs develop typical cartilage lesions in this period), statistical differences were presented with blue * (P<0.05) and † (trend). HA treatment reduced urinary Col2Ctcs at one month after the last injection - red * (Figure 2).

Figure 2. Normalized urinary Col2Ctcs. (* P < 0.05, † 0.05 < P < 0.1)

Conclusion: The results indicate an increased bone remodeling accompanied with superficial to middle layer lesions in the 11-month-old spontaneous guinea pig OA. We conclude that early bone remodeling plays a fundamental role in the development of osteoarthritis.

Hyaluronan treatment did not show a structural modifying effect when assessed by cartilage morphological analysis, however the suppression of urinary Col2Ctcs by HA at later time points suggests that it has a partial chondroprotective effect in this model.

References:

** University of Washington, Seattle, WA, USA
*** McMaster University, ON, Canada

swang@omers.com