Close Window

Print

**Submitted**

on June 18, 01:57 PM

for mrsfall2009

**Proof**

**CONTROL ID:** 668310

**CONTACT (NAME ONLY):** Fei Liang

**Abstract Details**

**PRESENTATION TYPE:** Oral Presentation Preferred

**SYMPOSIUM:** QQ: Responsive Gels and Biopolymer Assemblies

**KEYWORDS:** Composition & Microstructure / Surface Techniques / scanning probe microscopy (SPM), Composition & Microstructure / Material Type / biological, Performance / Material Form / nanostructure.

**Abstract**

**TITLE:** Single Molecule Structure and Properties of Human Intervertebral Disc Aggrecan.

**AUTHORS (FIRST NAME, LAST NAME):** Fei Liang[1], Peter Roughley[6], Alan Grodzinsky[2, 3, 4], Christine Ortiz[5]

**INSTITUTIONS (ALL):** 1. Chemical Engineering, M.I.T., Cambridge, MA, USA.
2. Biological Engineering, M.I.T., Cambridge, MA, USA.
3. Electrical Engineering and Computer Science, M.I.T., Cambridge, MA, USA.
4. Mechanical Engineering, M.I.T., Cambridge, MA, USA.
5. Materials Science and Engineering, M.I.T., Cambridge, MA, USA.
6. Genetics Unit, Shriner's Hospital for Children, Montreal, QC, Canada.

**ABSTRACT BODY:** The intervertebral disc (IVD) is a fibrocartilaginous tissue located between the vertebrae in the spinal column. The large self-assembling proteoglycan, aggrecan, within the IVD extracellular matrix (ECM) is essential for resisting multiaxial compressive loads during physiological activity. Aggrecan degradation, resulting from abnormal and/or reduced cellular synthesis as well as proteolytic cleavage, leads to a reduction in disc biomechanical functionality and clinical pathology. Knowledge of the molecular-level structure and properties of aggrecan at different stages of degradation can help provide a fundamental mechanistic understanding of the disc degeneration process. This study utilizes high resolution tapping mode atomic force microscopy (AFM) to directly visualize the single molecule structure of aggrecan before and after removal of keratan sulfate (KS) or chondroitin sulfate (CS) glycosaminoglycans (GAGs) constituents via enzymatic treatment with Chondroitinase ABC (CSase) and Keratanase II (KSase), respectively. Aggrecan was extracted and purified from the healthy intervertebral disc of a 24-year old human and separated into two aliquots as follows: 1) a pool composed of aggrecan that were originally attached to hyaluronic acid via their G1 end domains in vivo ("aggregated"); and 2) a pool consisting of aggrecan that lack G1 domains and were not associated with hyaluronic acid in vivo and instead free in the ECM ("non-aggregated"). For the aggregated aggrecan samples, AFM height images showed that, on average, the contour length of the core protein (Lc) for the CSase treated molecules (Lc = 118 ± 65 nm, n (number of molecules) = 98) was 26% shorter than the untreated (Lc =184 ± 125 nm, n = 176).The aggregated KSase treated Lc aggrecan (166 ±106 nm, n =161) was found to be 10% shorter compared to the untreated aggregated aggrecan. Similar trends were observed for the non-aggregated pool. These data suggest that the core protein of the aggrecan is extended by the presence of the GAG chains, especially the CS-GAGs, presumably due to repulsive electrostatic double layer and steric intra- and intermolecular forces. Aggregated aggrecan was found to be relatively longer (20%) then that in the non-aggregated pool, indicating that molecules in the latter pool are likely degradation products entrapped in the extracellular matrix. Compared to human articular cartilage (Lc = 216 ± 10 nm, n = 193, obtained from 29-year old human being), the aggrecan in human IVD appeared to be more degraded. In addition, very few full-length aggrecan (possessing both G1 and G3 domains) was observed in the disc specimens, which fact further confirms that the disc aggrecan is comparatively more degraded then articular cartilage aggrecan. A higher degree of degradation in the disc aggrecan poses a larger challenge for the disc repair through clinical methodologies such as tissue engineering.

(No Table Selected)