**3D Finite Strains on the Cellular Level in Bovine Annulus**

Corinne J. Jongeneelen, Yvonne Schroeder, Famke Kraaijeveld, Jacques Huyghe

Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands

y.schroeder@tue.nl

**Introduction:** Intervertebral disc tissue consists of a fluid-filled extra-cellular matrix, in which living cells are sparsely dispersed. The mechanical function is highly dependent on the composition of the extra-cellular matrix, which primarily consists of collagen fibrils and negatively charged proteoglycans. Due to the fixed charges of the proteoglycans (PGs), the cation concentration inside the tissue is higher than physiological. This excess of ion particles leads to an osmotic pressure difference, which causes swelling of the tissue [1]. It has been shown that the osmotic pressure inside cartilaginous tissues is much higher than would be expected based on its FCD [2]. This is because part of the water in the tissue is absorbed by the collagen fibers. The proteoglycan molecules, because of their large size, are excluded from this intra-fibrillar space. This means that their effective concentrations are much higher in the extra-fibrillar space than if they were distributed uniformly throughout the entire matrix. Hence, the effective fixed charge density is higher than if computed from total tissue water content. A recent study demonstrates that intrafibrillar water increases osmolarity within the annulus fibrosus substantially [3].

On the other hand, Wognum et al. [4] showed by means of a physical and a numerical model of the disc that high osmolarity within the disc has a protective effect against crack propagation within the disc. Furthermore, experimental results have shown that gene expression of cells is affected by extracellular osmolarity changes [5]. Hence, a closer evaluation of osmolarity changes on deformations of the extracellular and pericellular matrix is needed. The purpose of this study is (1) to measure 3D finite strain in the micromechanical environment and (2) to demonstrate the feasibility of this method to grasp swelling strains on the cellular level.

**Materials and Methods:** Bovine tail discs are dissected and snap frozen in -80 degrees Celsius. The annulus tissue are sliced in 1 mm thick samples perpendicular to the fiber direction and placed into phosphate buffered saline to hydrate for 2 hours. The collagen is stained using Oregon green labeled CNA35 [6], while the cells are visualized using Propidium Iodide. The samples are submersed in 0.15 M NaCl solution and placed in a Zeiss LSM 510 CLSM. The sample is covered with a dialysis membrane (MW cutoff 8000) above which a solution of 0.15 M NaCl and polyethylene glycol (PEG) is applied. The temperature is controlled at 25 ± 1 degrees Celsius. The PEG concentration is set at 10 g/100 g, which amounts to a compressive prestress of about 0.1 MPa [7]. 3D image correlation [8] is used to reconstruct the three principal Green–Lagrange strains.

**Results:** The CNA35 probe visualizes a highly organized and heterogeneous collagen network in the annulus. By means of cell visualization with PI nuclear stain cell stretching perpendicular to the fiber direction is noticed, as well as rotational deformations. The results show the heterogeneous character of the tissue as well as the non-affine nature of the deformation, particularly around clefts and cells. (Fig. 1)

**Discussion:** The micro deformation under osmotic loading of the annulus tissue is strongly heterogeneous. The 3D correlation technique calculates 3D strain in collagen rich areas and on the cellular level. The preliminary results indicate that strain changes as a function of distance from the cell.

Also changes in osmotic prestressing may induce high micro strains around clefts and hence stress concentrations. These stress concentrations cannot possibly be inferred from a finite element analysis that deals with average strains and stresses only. Duncan and coworkers have demonstrated shearing of collagen fibrils in the bovine disc [9]. The present results indicate large normal strain between the fibrils on top of shearing. Around cells the opposite seems to happen strains tend to increase as we move away from the cells, suggesting a protective function of the pericellular matrix (Fig. 1).

**References:**