CELL DEFORMATION IN RESPONSE TO LOCAL MATRIX STRAIN

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INTRODUCTION

Chondrocyte deformation within compressed extracellular matrix (ECM) and/or agarose composites has been studied extensively during the past years [e.g. 1,2]. Since the elastic modulus of the cells is some magnitudes smaller than that of ECM, a stress concentration exists on the micro-level around the chondrocyte [3]. This needs to be taken into consideration for tissue engineering because the initial elasticity modulus of the cell-seeded artificial matrix is typically lower than that of mature ECM. In order to evaluate stresses and strains at the micron-level numerical models may be used. For the input a precise description of the matrix deformation in the immediate vicinity of the cell is necessary. In this study we employed a technique based on grayscale correlation of microscopy images. We hypothesized that considerable strain perpendicular to the primary loading axis occurs and strains are affected by cell type.

METHODS

Metacarpalphalengeal joints from 18–months old steers were dissected to obtain samples from articular cartilage. Superficial (~15% uppermost) and deep (rest) zones were separated by slicing manually. Chondrocytes were isolated by digesting the slices with pronase and collagenase. Isolated cells were labeled with calcein dye and cell/agarose constructs were prepared by mixing $5.7x10^6$cells ml $^{-1}$ of 3% agarose. In addition, fluorescent beads (molecular Probes) were added as extracellular markers. After solidification at 4ºC, constructs were cut into ten 10mm cubes each. A loading apparatus (Fig. 1) was used to apply unconfined compression in steps of 500 microns up to 2 mm. The corresponding local agarose deformation was studied using a confocal laser microscope capturing the relative displacement of the fluorescent beads. The sets of images were analyzed using VEDDAC™- an image correlation software which uses grey scale data to give a vectorial representation of displacement. Creating a grid of measurement points with an X & Y pitch of 65µm, the grey scale information from a 4350µm^2 correlation area around each point was used to identify the displacement. The search area was limited to 33000 µm^2. A strain calculation algorithm based on linear regression was added to determine the local principal strains. The deformation index (Fig.1) and perimeter of cells were quantified for ten cells per cube and load step.

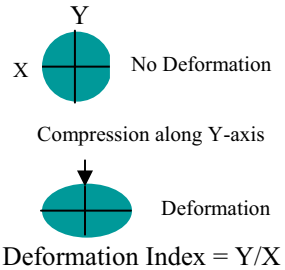

Figure 1: Compression apparatus

RESULTS AND DISCUSSION

A homogenous gradient of deformation was observed for all samples both in axial and lateral direction (Fig.2). On average, the local axial strain was similar to applied 'global' strain, however, individual values varied greatly among samples as well as between deformation steps. Thus, a nominal 5% step generated local strains from as low as 1.3% to as high as 8.8%. The local lateral strain was dependent on applied load and decreased with increasing axial strain (Fig.2) Thus, the Poisson-like values started from 0.45 and decreased with increasing strain to 0.26 and 0.32 for the superficial and deep cell-agarose composites respectively. Also deformation on the cellular level differed between the two cell populations. The slope of the deformation index was steeper for the deep cells (Fig.3). Interestingly, the cells behaved differently with regard to their perimeter (Fig.3) suggesting a growing cell volume for the deep cells and a shrinking volume for the superficial cells with increasing strain (Fig.3).

CONCLUSIONS

Locally generated strains were variable among samples and appeared to be affected by cell type and loading. Grayscale correlation is helpful in the precise computation of local strain.

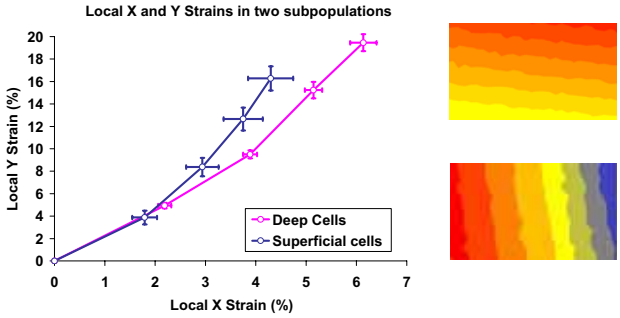

Figure 2: Local axial (Y) and lateral (X) strains for deep and superficial cell agarose constructs. Mean ± S.E., N=10. Contours depicting the local Y and X deformation gradients

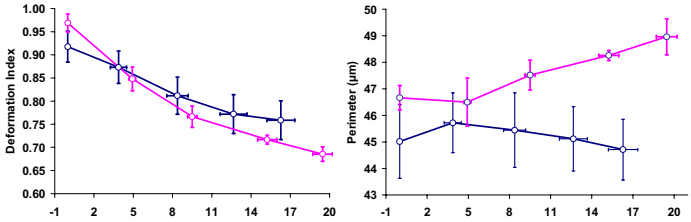

Figure 3: Deformation Index and Perimeter as a function of Local Strain for Deep and Superficial cells. Mean ± S.E., N=10. X axis = Local Strain (%)

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