The Role of Indirect Mechanotransduction Phenomena in Microtrauma Development within Intervertebral Discs – A Computational Biophysical Analysis

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I. INTRODUCTION

The causes of intervertebral disc (IVD) ruptures continue to elude both biomechanists and clinicians. It is widely accepted that unique traumatic high-load impacts are unlikely to lead to isolated IVD herniation. Therefore, a suggested mechanism for disc rupture is an accumulation of microtrauma within the IVD tissue. Microtrauma development can be related to tissue fatigue caused by repetitive (physiological) load application, as shown, for example, in [1]. However, the impact of long-term biological influence on microtrauma, such as changes in the tissue quality due to cellular activity, has not yet been extensively addressed. A persistent catabolic shift of cellular activity possibly leads to a reduction of the tissue's capability to resist loads. Mechanobiological investigations showed that loads acting on the tissue impact cellular predisposition and therefore influence tissue maintenance (e.g. [2]). Loads can be sensed by cells in a direct or an indirect manner. Direct mechanotransduction refers to loads transmitted over the extracellular matrix (ECM) directly on the cell's membrane, whereas indirect mechanotransduction refers to alteration in the ECM compaction, changes in solute transport to the cells and the effect of this on cell behaviour [3]. We assume that the latter is of special interest with regard to IVD microtrauma emergence because nutrition of Nucleus Pulposus (NP) and inner Annulus Fibrosus (AF) cells is diffusion-dependent. The objective of this project is to find crucial mechanisms at the tissue and cellular levels that lead to microtrauma within the IVD. This short paper presents the first results to address the influence of indirect mechanotransduction on the predisposition of NP cells to develop catabolic activity and contribute locally to IVD tissue damage.

II. METHODS

A 3D Agent-Based Model (ABM) of 4,000 NP cells within 1 mm^3 was simulated. The ABM world was structured as a concentric system of patch-sets (cubes) through which a numerically calculated amount of solutes, namely glucose (glc), lactate (lac) and oxygen (O_2), are diffused, depending on the porosity of the medium (Fig. 1). Alterations in boundary concentrations (BC) of solutes due to indirect mechanotransduction were extracted from poroelastic Finite Element (FE) mechanotransport calculations [4], in which the cartilage endplate (CEP), through which solutes enter the IVD, was simulated as normal or degenerated. It was chosen to simulate the outermost anterior region of the NP as the highest nutritional stress could be observed in this region [5]. Cells metabolised O_2 and lac according to empirical equations [6]. Glc was estimated based on lac accumulation, whereby a completely anaerobic cell metabolism was assumed. Acidity was estimated supposing a linear relationship between pH and lactate accumulation [7]. O_2 / glc availability and pH were calculated every hour for each cube. To estimate cell viability and mRNA expression according to the current solute availability, continuous mathematical interpolations of sets of discrete measurements of in vitro cell cultures were built.

TABLE I
BC FOR THE ABM: 1. CEP NOT DEGENERATED, 2. CEP DEGENERATED

	1.	2.
Glc [mM]	1.03	0.85
Lac [mM]	4.92	5.23
O_2 [kPa]	1.10	0.98

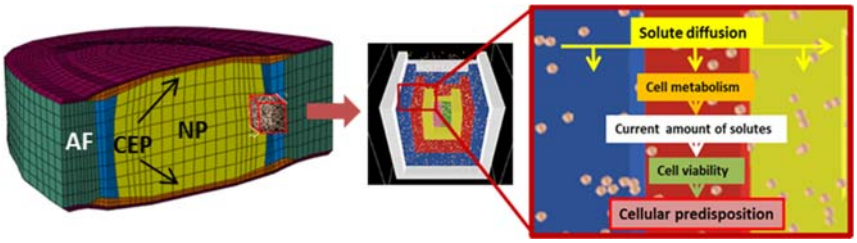

Fig. 1. Left: region of interest within FE model. Middle: ABM with highlighted cube system. First cube: white, second cube: blue, third cube: red, fourth cube: yellow, fifth cube: green/rose. Right: process of estimation of cellular predisposition for each cube.

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To estimate cellular predisposition, these interpolations were integrated using a dynamic Boolean-based network, which allows a weighting of the influence of each solute on mRNA production [8]. The mRNA expression of the main ECM proteins, Aggrecan (Agg) and Collagen II (Col-II), the key ECM degrading protease families MMPs and ADAMTS and of Collagen I (Col-I) was simulated. Cells were exposed for 4h to solute levels calculated with and without CEP degeneration (Table I). A two-factor ANOVA was used for statistical analysis.

III. INITIAL FINDINGS

Initial findings indicate decreased mRNA expressions of Agg and Col-II and an upregulation of MMP mRNA due to CEP degeneration. An upregulation of Col-I mRNA was also indicated (Table II). Differences in mRNA expression due to indirect mechanotransduction phenomena are highly significant for each type of mRNA at each cube ($p<0.001$).

TABLE II
RELATIVE CHANGE OF MRNA PRODUCTION UNDER IMPAIRED SOLUTE AVAILABILITY

Cube	Agg	Col-I	Col-II	MMP	ADAMTS
First	-0,0110	0,0464	-0,0268	0,0514	0,0021
Second	-0,0109	0,0472	-0,0265	0,0518	0,0022
Third	-0,0107	0,0486	-0,0261	0,0524	0,0024
Fourth	-0,0106	0,0502	-0,0256	0,0531	0,0029
Fifth	-0,0107	0,0486	-0,0261	0,0524	0,0037

IV. DISCUSSION

First results indicate a significant catabolic shift of cellular activity within the NP due to indirect mechanotransduction phenomena: cells produce between 1% and 3% less ECM protein mRNA and at the same time express up to 5% more protease mRNA. Significance was reached due to small standard deviations within this generic model. However, relative changes in mRNA expression are objectively small, but considering accumulative effects due to long-term exposure of cells to solute alterations, the significance of these findings increases. This simulated catabolic shift on cellular activity is in accordance with observed tissue changes due to degeneration [9]. Likewise, an augmentation of Col-I mRNA is consistent with observations that in degenerated IVDs, total amounts of collagen remain constant whereas collagen types may alter [2],[9]. However, the most pronounced change of NP tissue composition is reflected by a loss of proteoglycans (Agg) [9]. Thus, this model may underestimate the shift in Agg mRNA expression. Still, it has to be considered that direct mechanotransduction phenomena were not yet included, which presumably will further influence mRNA expression. Moreover, complex interactions between individual influences of solute concentrations on mRNA production and feedback loops with inflammatory cytokines need to be further addressed for upcoming model development in order to improve quantitative estimations of mRNA expression. Nevertheless, our initial findings suggest a considerable influence of indirect mechanotransduction phenomena on cellular activity, which suggests a need to consider more explicitly the biological factors in microtrauma evolution.

A further step in this project includes an adaptation of the model to simulate the cellular activity within the AF. Obtained information about regional changes on tissue quality will be coupled with a detailed FE model that simulates AF ultrastructures in order to investigate microtrauma emergence over multiple scales.

V. REFERENCES

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