Mechanical Effect on Cell Viability in Healthy and Degenerated Intervertebral Discs

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Introduction

The study of cell death within the intervertebral disc (IVD) enables to understand better the process of disc degeneration. Recently, a model simulating the coupling between mechanical loading and the transport of metabolites has shown the effect of mechanics on transport [1]. However, modelling the link between solute/metabolism concentrations and cell activity is still an open issue. In the literature it has been shown how the effect of acidic pH (due to lactate accumulation) can be detrimental to cell viability [2,3,4]. Also, glucose has been underlined as the most relevant nutrient affecting cell viability: Horner and Urban [3] have found that cell concentration in a diffusion chamber decayed exponentially over time below critical glucose concentration and pH values. Accordingly, cell viability criteria in a metabolic transport IVD FE model have been introduced [2]. However, disc mechanics effects over time have not been considered so far. Thus, our first aim was to close this gap and determine whether tissue deformation effects on metabolic transport would affect cell death within the IVD. Then, our second aim was to characterize the outcome of the interactions among disc mechanics, metabolic transport, and cell death depending on tissue mechanical and biological properties in both healthy and degenerated conditions.

Materials and Methods

A coupled IVD FE mechano-transport model [1] was modified to include pH- and glucose-dependent cell viability criteria. To verify such criteria, a diffusion chamber was simulated according to experimental tests on bovine nucleus cell viability [3]. A 26 mm width diffusion chamber filled with cells embedded in 1% agarose gel was modelled. Oxygen, lactate and glucose diffusivities accounted for gel porosity. Only half of a thin slice was reproduced due to chamber geometry. Lactate accumulation, and oxygen and glucose consumption were interrelated and followed metabolic rates found *in vitro* on bovine nucleus pulposus (NP) cells [4]. Initial conditions were applied throughout the chamber for the three metabolites to reproduce the experiment: an initial pH of 7.4 (i.e. an initial null lactate concentration), an initial oxygen pressure of 21 kPa, and an initial glucose concentration of 5 mM. During simulation, such values were maintained only at the boundary since nutrient concentrations were maintained in the medium along the experiment [3]. Cell viability was considered by modifying the cell density ρ_{cell} over time, depending on the glucose and pH levels predicted. Based on the experimental results of Horner and Urban [3], cell death rates were represented by exponential functions of the type:

$$\rho_{cell} = \rho_{cell,0} \exp(-\alpha_i t) \qquad \alpha_i = 9.28\times10^{-6} \text{ for } i = glucose \text{ and } \alpha_i = 3.43\times10^{-6} \text{ for } i = pH.$$

The exponential decays were assumed to start when glucose and pH felt below two critical values. Such "threshold" values that initiated cell death were adjusted to reproduce realistically the temporal and spatial in vitro viability curves as a function of cell density determined in the above-cited experiment [3].

By using the fully coupled IVD model [1], a daily compression (16h standing and 8h resting) was considered. Metabolite concentrations at the disc boundaries corresponded (i) to a normal blood supply (NBS) or (ii) were reduced by 50% of such reference values (RBS). Cell densities were updated at each integration point by considering the exponential decays as well as the volume changes

when mechanical deformations were coupled. Degeneration was simulated by decreasing IVD height, subtissues fluid contents and initial cell densities and by increasing solid-phase stiffness [1]. A preconditioning of 2-days for metabolite diffusion was applied prior to consider mechanical coupling and viability. Solutions obtained with or without mechanical couplings for both degenerated and healthy properties were compared.

Results

In Fig. 1, viability profiles predicted by the model are shown in comparison to the reference experiment, for different initial cell densities, at two time lapses (3 and 6 days of culture). In terms of viable distance at which cells started to die, there was a good agreement of the computed cell viability profiles against literature data. However, the shapes of viability curves matched better the experimental ones at day 6, especially at higher initial densities. The adjusted threshold critical values of glucose and pH were 0.5 mM and 6.8, respectively.

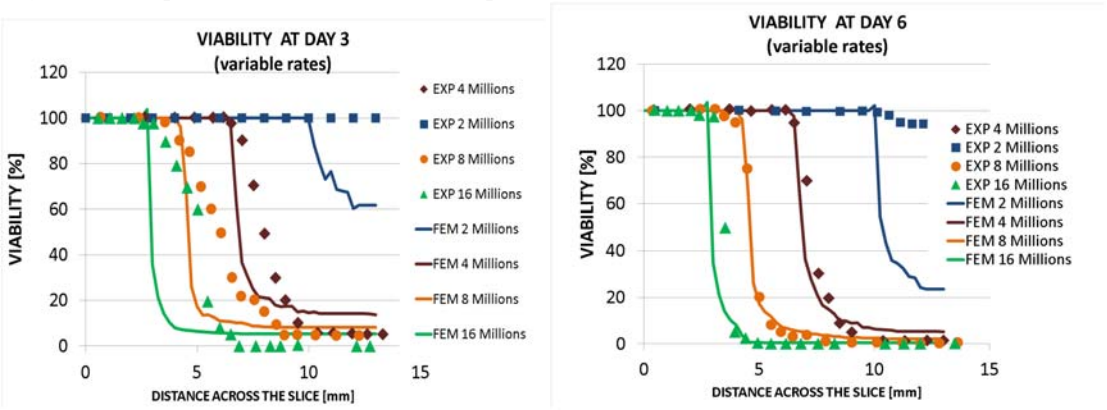

Figure 1: Viability profiles in the simulated half-slice of the chamber and their comparison with experimental results [3], for different cell density (in millions cells/mL). Numerical results from FEM computed and compared at day 3 (left) at day 6 (right).

For the IVD model, NBS condition maintained glucose and pH levels above the critical ones. In case of RBS (Fig. 2) cells started to die in the IVD centre, since critical glucose concentrations were reached. Deformation couplings increased glucose concentration and therefore cell viability. As a consequence, cells stopped dying about 10 hours earlier when mechanical deformations were considered. Also, it was possible to compare the spatial distributions of cell death rate at the end of the simulated period with or without mechanical couplings for both healthy vs. degenerated disc properties (Fig. 3). For healthy properties the most evident change occurred in the central disc, where mechanical deformations increased the glucose concentrations and therefore cell viability was higher. However, mechanical couplings also decreased cell viability in the anterior inner annulus fibrosus due to the prevalence of a strain-dependent effect that reduced the diffusivity in this area. In the degenerated IVD the mechanical effect was negligible (Fig. 3).

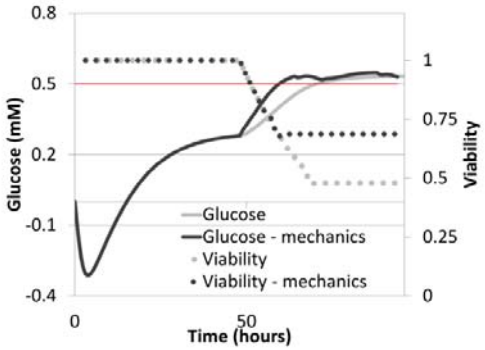

Figure 2: RBS case: Glucose and viability in the IVD centre with and without mechanical coupling. The first 48 hours refer to preconditioning.

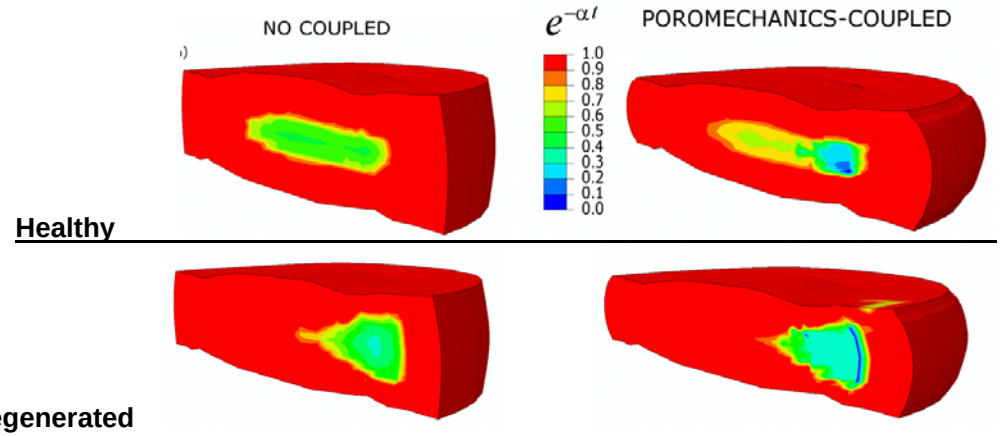

Figure 3: Distributions of the cell viability exponentials $\exp(\alpha_{glucose}t)$ (fraction of surviving cell density) computed at the end of the 16-hour creep of the second day simulated, with (left) and without (right) poromechanical coupling, and for healthy (top) and degenerated (bottom) IVD properties.

Conclusions

Deformability of healthy IVDs was found to be positive for the maintaining of cell viability in the disc centre. Moreover, in degenerated discs cell death acceleration may occur due to the loss of compliance that hinders proper metabolic transport. Our novel mechanobiological study of the intervertebral disc points out on the restoration of mechanical properties as a potentially beneficial regenerative treatment for cells in those cases where a reduced nutritional balance at the disc boundaries can occur.

Acknowledgements

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