

Cartilage Endplate Permeability: From *In Vitro* to *In Silico*

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ABSTRACT INTRODUCTION:

Studies have shown that Intervertebral Disc Degeneration (IDD) can result from chemical changes in the Cartilage Endplate (CEP)¹, implying that pain is closely related to CEP weaknesses and imperfections². This was further evidenced by studies revealing nerve fiber and blood vessel infiltration into the Intervertebral Disc (IVD) not only through the annulus fibrosus, but also through the CEP. This infiltration is a major cause of lower back pain (LBP)¹. Yet, among all the spinal tissue, the CEP receives the least amount of attention in scientific literature. In computational studies, the CEP is described in very generic terms e.g. as a 1D theoretical model or even reduced to a boundary condition of the IVD, despite its crucial role in keeping the IVD healthy by acting as the main gateway of nutrients and waste in and out of the avascular region³.

The purpose of this study is to follow a combined *in vitro* and imaging-driven *in silico* approach to obtain an improved understanding of the CEP functionality regarding mass transport. The study is divided into three objectives: 1) ex-vivo CEP fluid dynamics determination; 2) nanofocus Computed Tomography (nCT) to generate image files and measurements; and finally, 3) 3D model construction and *in silico* modeling validated by *in vitro* studies.

METHODS:

***In vitro*:** A fresh bovine tail was obtained from the local slaughterhouse. 6 CEPs and 2 vertebral bodies (VBs) were harvested from it. Eight mm diameter biopsy samples were prepared and enabled to free swell prior to testing. Two types of fluid were used, Phosphate Buffer Saline (PBS), and Dulbecco's Modified Eagle Medium (DMEM), which is a commonly used culture medium supplemented with 10% Fetal Bovine Serum and 1% Antibiotics and Antimycotics. Each sample was fitted into a silicone tube tightened with a ring clamp open to the atmosphere at one end and connected to a fluid source with a constant level at the other end ensuring constant pressure around the sample. Testing on each sample was performed twice in the forward and reverse directions for 10min per run and the amount of passed fluid was collected to determine the flow rate. The test was performed in both directions to account for the fact that the CEP properties are influenced by the chemical components and might not be the same in both directions.

***In silico*:** The samples were incubated in 40% contrast agent Hexabrix for 48 hours at room temperature. The samples were then imaged by a Nanotom® M (GE nanoCT©) using in-house optimized settings for osteochondral tissues with a resolution of 4µm. From the reconstructed nCT images, 1 mm diameter sections were virtually punched out to create 3D models of the pore structure in the CEPs. The models were then manually curated to generate a high-quality volume meshes of the CEPs using Blender and Gmsh. Subsequently, the model was imported into OpenFOAM® to perform Computational Fluid Dynamics (CFD) Simulations using incompressible laminar flow equations to quantify the mass transport through the CEP. The inlet and outlet were taken as the bottommost and uppermost layers of the mesh while all other layers were assigned as walls with a no-slip condition. The initial pressure and flow rate were same as the experimental results. The viscosity was determined from literature reviews. The simulation ran for 20 sec and sampled every 0.1 sec.

RESULTS SECTION:

From fluid flow testing, the VB showed a relatively high volumetric flow rate compared to the CEP (average of 2.2 ml/min), which is an order of magnitude higher than that of the CEP (average of 0.4 ml/min) (Fig.1) and was thus ignored in the CEP permeability analysis. The thickness (height) and porosity of the samples calculated from the nCT images averaged around 0.5 mm and 6.4% respectively. The permeability of the samples was calculated using Darcy's law, considering proper fluid viscosity, and averaged at a mean of $0.04 \pm 1.6 \times 10^{-2} \mu\text{m}^2$ and $0.1 \pm 5 \times 10^{-2} \mu\text{m}^2$ for DMEM and PBS respectively. Part of this permeability difference could be due to damage after repeated handling. That damage was visible especially in sample 5 (forward and reverse) and 6 (forward) where PBS tests were discarded. The *in silico* models have been created (Fig.2) and the CFD simulations are currently ongoing.

DISCUSSION:

The combination of image-based *in-silico* modelling with *in-vitro* experiments, as proposed in this study, is a first step towards a better quantification of the mass transport across the CEP, leading to an improved understanding of mass transport control in and out of IVDs through the CEP. As a limitation, it should be mentioned that the study is still in an early phase and that at this stage it does not account for changes of properties under loading, which is known to affect mass transport through the CEP, and has been completed on bovine CEP which are likely to show variation from human CEPs. It should be noted that using bovine tails as the animal models was chosen because of its similarity to human IVDs in physico-chemical properties and glycosaminoglycans (GAGs) content, especially at the proteoglycan-rich territorial matrix and collagen-rich interterritorial matrix at the center¹.

SIGNIFICANCE:

It is widely known that lumbar disc degeneration accounts for 50% of lower back pain in young adults. Yet, due to its highly multifactorial etiology, a patient stratification tool has not yet been created. A better understanding of the mass transport through the healthy and degenerated cartilage endplate would be an important step towards the creation of such a tool that would anticipate the risk of developing lower back.

REFERENCES:

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ACKNOWLEDGEMENTS:

This project received funds from the European Commission (H2020-MSCA-ITN-ETN-2020-955735). The micro (or nano)-CT images were generated on the X-ray computed tomography facility of the Department of Development and Regeneration of the KU Leuven, financed by the Hercules Foundation (project AKUL/13/47).

FIGURES:

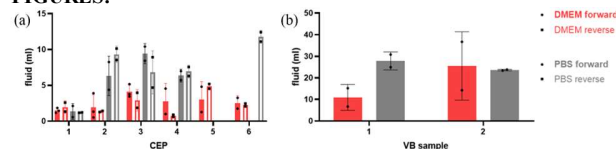


Fig. 1: Perfusion measurements. Volume passively passed through (a) bovine cartilage endplate samples and (b) bovine vertebrate bodies (8 mm diameter) in a 10 minute interval. The perfusion experiment was executed in both directions

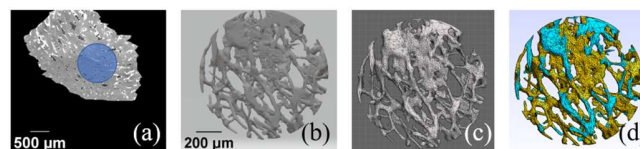


Fig. 2: Nanofocus Computed Tomography imaging of the cartilage endplate (CEP). (a) reconstruction of the CEP (b) 1 mm diameter virtual punch showing the CEP pores (c) generation of a computational mesh using Blender and Gmsh (d) manually curated mesh