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Osmosis and viscoelasticity both contribute to time-dependent behaviour of the intervertebral disc under compressive load: A caprine in vitro study

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ABSTRACT

The mechanical behaviour of the intervertebral disc highly depends on the content and transport of interstitial fluid. It is unknown, however, to what extent the time-dependent behaviour can be attributed to osmosis. Here we investigate the effect of both mechanical and osmotic loading on water content, nucleus pressure and disc height. Eight goat intervertebral discs, immersed in physiological saline, were subjected to a compressive force with a pressure needle inserted in the nucleus. The loading protocol was: 10 N (6 h); 150 N (42 h); 10 N (24 h). Half-way the 150 N-phase (24 h), we eliminated the osmotic gradient by adding 26% poly-ethylene glycol to the surrounding fluid. For 62 additional discs, we determined the water content of both nucleus and annulus after 6, 24, 48, or 72 h. The compressive load was initially counterbalanced by the hydrostatic pressure in the nucleus. The load forced 4.3% of the water out of the nucleus, which reduced nucleus pressure by 44(±6)%. Reduction of the osmotic gradient disturbed the equilibrium disc height, and a significant loss of annulus water content was found. Remarkably, pressure and water content of the nucleus pulposus remained unchanged. This shows that annulus water content is important in the response to axial loading. After unloading, in the absence of an osmotic gradient, there was substantial viscoelastic recovery of 53(±11)% of the disc height, without a change in water content. However, for restoration of the nucleus pressure and for full restoration of disc height, restoration of the osmotic gradient was needed.

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1. Introduction

The intervertebral disc connects adjacent vertebrae, transfers loads and provides flexibility to the spine. The main loading direction is axial (Smit, 2002), and the daily loading of the spine reduces water content and disc height, which is restored at night (Botsford et al., 1994; Emanuel et al., 2015).Osmotic attraction of water is considered to play an important role in both withstanding daily loads and the disc height recovery at night (Schroeder et al., 2006). With aging, however, the delicate homeostasis in the disc gets disturbed, leading to degeneration. This is a process in which cell biology, matrix physiology and biomechanics influence each other in a vicious circle (Vergroesen et al., 2015). This has been shown to be the major contributor to low-back pain (Cheung et al., 2009; Livshits et al., 2011), the most disabling disease worldwide (Vos et al., 2013). As the loss of charged proteoglycans in the nucleus pulposus that drive the osmotic gradient is considered the first sign of degeneration (Buckwalter, 1995), it is important to understand the role of osmosis in deformation and recovery. Furthermore, investigation of the interaction between osmotic, mechanical and hydrostatic pressure can provide valuable input for spine models, which are used for numerous tasks (Schmidt et al., 2013).

The current view on the role of fluid flow in the mechanical properties of intervertebral disc has been grounded in the late 70's by pioneering work by Urban and Maroudas (Urban and Maroudas, 1979, 1981; Urban and McMullin, 1984). They hypothesized that the negative charges in the proteoglycan chains stimulate an influx of positive ions. The increase in ion concentration results in a higher osmotic potential in the disc. This attracts water

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into the disc, thereby building up pressure. This special form of osmosis is called the Gibbs-Donnan effect (Elkin et al., 2010). When an external force is exerted on the disc, water is slowly pushed out of the disc. The velocity of the loss of water depends on the pore size of the matrix. The loss of water increases the osmotic pressure, as the ions are electrostatically bound to the proteoglycans and therefore remain in the disc. This continues until the osmotic pressure in the disc is in balance with the external pressure. Although the model is appealing, it is well-known that multiple time-dependent processes are occurring during loading and unloading of the intervertebral disc. Viscoelastic properties of the collagen fibres are likely contributing to deformation of the disc as well (Shen et al., 2011; van der Veen et al., 2013). However, it is difficult to separate the processes experimentally. The most straightforward way to do this would be to selectively eliminate one of these processes. Some studies tried to reduce the osmotic gradient by injecting substances that break down the proteoglycans (Detiger et al., 2013), but this also affects the hydraulic permeability of the tissue (Johannessen and Elliott, 2005). Another way to eliminate osmosis is to increase the osmolality of the fluid surrounding the disc. The aim of this study is to assess the role of osmosis in the biomechanical behaviour of the intervertebral disc. Therefore, we apply a simple loading protocol of static compression and subsequent unloading. When in equilibrium with the load, we will largely eliminate the osmotic gradient to visualize the effect of this gradient on the intradiscal hydrostatic pressure, disc height and water content of the nucleus pulposus and annulus fibrosis. Subsequently, the load is released without restoring the osmotic gradient. We hypothesize that the pressure, disc height and water content will decrease when the osmotic gradient is reduced, and that limited recovery is seen after unloading.

2. Methods and materials

Eighty-four lumbar intervertebral discs (13 T13-L1; 16 L1-L2; 13 L2-L3; 14 L3-L4; 13 L4-L5; 13 L5-L6, average transversal disc area $4.35 \pm 0.46\ cm^2$) were dissected from fresh-frozen mature caprine spines (age: 3–5 years) by parallel cuts through the vertebral bodies. Approximately 3.5 mm bone remained on each side of the discs, making sure that the entire disc is intact. The bone was rinsed and cleaned and inserted in a chamber filled with standard physiological saline (0.9% or 0.15 mol/l NaCl; Osmotic pressure: 731 kPa calculated using Eq. (1). Titanium porous platen on top of the endplates ensured that the fluid was completely surrounding the disc.

$$\Pi = c * R * T \tag{1}$$

With Π is osmotic pressure in kPa, c is the molar concentration in osm/l, R is the gas constant and T is the temperature in K.

2.1. Intradiscal pressure measurements

For eight intervertebral discs, the mechanical load was applied using a biomechanical testing device (model 8872; Instron and IST, Norwood, Canada) which allows precise application of the compressive load, as well as measurement of the changes in disc height by monitoring the displacement of the testing device. Furthermore, the hydrostatic pressure in the nucleus was measured using a 1.33 mm pressure needle (CTN-4F; Gaeltec devices Ltd, Dunvegan, Isle of Skye, Scotland), inserted through the lateral annulus until the tip of the needle was in the middle of the disc (see Fig. 1). All experiments were conducted in a temperature-controlled room at 20 deg Celsius. At t = 0, the discs were submersed in physiological saline, and an axial preload of 10 N was applied (see Fig. 2). At t = 6 h, the load was increased to 150 N,

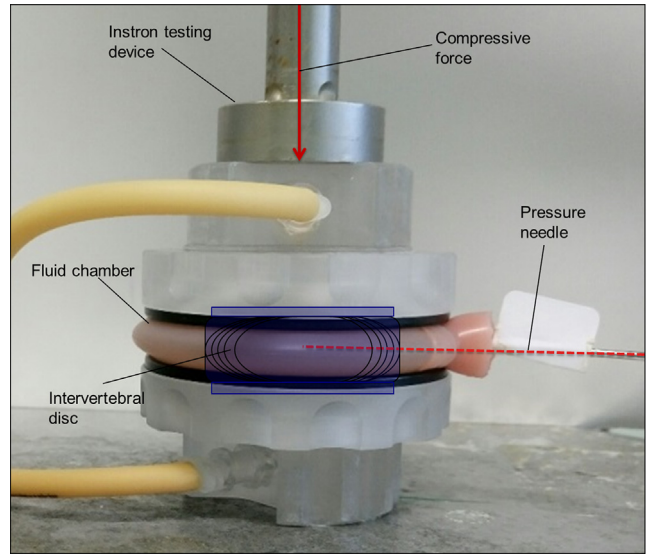

Fig. 1. Experimental setup.

which is estimated to be within a typical range for a goat during daily activities. 150 N is approximately 0.34 MPa, if the whole disc area is considered to carry the load. This static loading was held for 42 h. At t = 24 h, the osmotic gradient across the annulus fibrosus was largely eliminated by dissolving 26.6% Polyethylene glycol (PEG) in the surrounding medium, thus increasing the osmotic pressure by approximately 0.7 MPa (Michel, 1983; Money, 1989). At t = 48 h, the compressive pressure was released to 10 N again for an additional 24 h, while the osmotic pressure was kept high.

2.2. Water content measurements

Sixty-two additional discs were subjected to the same loading regime using a custom-build loaded disc culture system, which allows the change of culture fluid and axial compressive loading. Samples for water content measurements were taken at selected locations (Fig. 3) at t = 6 (n = 11), t = 24 (n = 17), t = 48 (n = 21) or t = 72 (n = 13) hours. After removal of the cranial end-plate with a surgical blade, a strip of approximately 5 mm wide was taken from side to side, and divided in 6 equal parts (see Fig. 3). The two outer sections (excluding the outermost lamella to avoid that wetness of the surface of the disc during dissection influenced results) will be referred to as annulus, the two inner sections as nucleus. The four tissue samples were removed with a blade and stored in a closed Eppendorfer tube with a pre-measured weight. Wet weight was measured with a Satorius scale (type R180D, Göttingen, Germany). After freeze-drying the samples with a speedvac, the dry weight was measured. Water content was calculated as shown in Eq. (2).

$$Water\ content = \frac{ww - dw}{ww} * 100 \tag{2}$$

With ww = wet weight (g) and dw = dry weight (g).

2.3. Extended protocol

To check whether the discs were still intact after the experiments and show complete recovery, in the final four discs the protocol was extended for an additional 24 h on norm-osmotic physiological saline. Also, for an additional 14 discs, water content was determined at t = 96 h after the extended protocol of 96 h.

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