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Fluid-flow dependent response of intervertebral discs under cyclic loading: On the role of specimen preparation and preconditioning

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ABSTRACT

In vivo during the day, intervertebral discs are loaded mainly in compression causing fluid and height losses that are subsequently fully recovered overnight due to fluid inflow under smaller compression. However, *in vitro*, fluid flow through the endplates, in particular fluid imbibition, is hampered possibly by blood clots formed post mortem. Despite earlier *in vitro* studies, it remains yet unclear if and how fluid flow conditions *in vitro* could properly emulate those *in vivo*.

Effects of various preload magnitudes (no preload, 0.06 and 0.28 MPa) and disc-bone preparation conditions (*e.g.*, w/o bony endplates) on disc height and nucleus pressure were investigated using 54 bovine specimens. Changes in specimen height and pressure at different nucleus locations were used as surrogate measures to assess the fluid content and flow within the discs.

Under all investigated preparation conditions and preload magnitudes, no significant pressure recovery could be obtained during low loading phases, even without bony endplates. On the contrary, partial to full displacement recovery were reached in particular under 0.28 MPa preload.

Results highlight the significant role of disc preload magnitude in disc height recovery during low loading periods. Attention should hence be given in future studies to the proper selection of preload magnitude and duration as well as the animal models used if *in vivo* response is intended to be replicated. Findings also indicate that flushing the endplates or injection of bone cement respectively neither facilitates nor impedes fluid flow into or out of the disc to a noticeable degree in this bovine disc model.

1. Introduction

The primary function of the intervertebral disc is mechanical; it supports and transmits loads from one spinal level to another while providing the spinal compliance required to perform various daily activities. Because of its high water content, ~85% in the nucleus and ~70% in the annulus (Urban and McMullin, 1988), and poroelastic nature, fluid flow plays a key role in its transient response and load transmission (Adams et al., 1987). *In vivo* over the course of a day, the disc fluid content continuously varies with time, depending on loading (arising from the upper body weight, external/inertial loads and muscle activation), posture and internal osmotic pressure (reflecting its composition) (Botsford et al., 1994; Dimitriadis et al., 2011; Malko et al., 2002; Nazari et al., 2015; Paajanen et al., 1994; Wing et al., 1991). As a result, the disc volume, height (Botsford et al., 1994) and nucleus pressure (Wilke et al., 1999) alter depending to a large extent on the disc fluid content and flow. Although the diurnal loading lasts on average nearly twice longer than the subsequent resting (16 *vs.* 8 h), the disc completely recovers its height and volume during the latter period through fluid inflow driven mainly by an osmotic pressure gradient favored by rather small external loads (Adams et al., 1990). The fluid movements occur mainly through marrow contact channels of the endplates (Cassidy et al., 1989; Nachemson et al., 1970; Ogata and Whiteside, 1981) in which fluid imbibition during unloading likely faces less resistance than fluid exudation in loading resulting in flow direction-dependent permeability of endplates (Ayotte et al, 2001). The fluid flow may also occur through the peripheral annulus, though at a lower rate (Ferguson et al., 2004; Nachemson et al., 1970).

The time dependent mechanical behavior of discs has extensively been investigated *in vitro* (Adams et al., 1987, 1990, 1996; Kraemer

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et al., 1985; Reitmaier et al., 2012a, 2012b; van der Veen et al., 2005, 2007; 2009). It remains however yet unclear if conditions *in vitro* properly emulate those *in vivo*. Several studies have raised concerns on the fluid flow through the endplates of being hampered post mortem by blood clots and bone debris that likely impede fluid flow *in vitro* (Adams and Hutton, 1983; Ayotte et al., 2000; Lee et al., 2006; MacLean et al., 2007; van der Veen et al., 2005). Studies on human, ovine, bovine, porcine, caprine and rat discs corroborate that the losses in the disc height and nucleus pressure under constant compression due to fluid exudation are not fully regained during subsequent recovery period upon unloading or low loading phases indicating an insufficient fluid inflow (Lee et al., 2006; Lin et al., 2009; O'Connell et al., 2011; Reitmaier et al., 2012a, 2012b; van der Veen et al., 2005, 2007, 2009).

In retrospective, however, systematic analyses of preconditioning and specimen preparation conditions on resultant fluid flow and recovery are lacking. In previous investigations, the duration of preconditioning differed considerably and was usually short without displacement equilibrium (Reitmaier et al., 2012a, 2012b; van der Veen et al., 2005, 2007, 2009). Furthermore, the magnitude of the preload was rather small when compared to that of the load applied in the subsequent loading phase. The progressive loss in the disc height and pressure under loading and the limited recovery in the disc height and absence of pressure gain during subsequent phases under much smaller loads may partly depend on these initial conditions. A longer preconditioning likely also restores more physiological initial conditions. Moreover, specimen preparations that involve for example flushing out blood clots and bone debris from endplates or removing adjacent bony structures along with the application of dynamic recovery loads may enhance fluid inflow and thus disc recovery *in vitro*.

Existing *in vitro* experiments measured the disc pressure only at the center of the nucleus (Reitmaier et al., 2012a, 2012b; van der Veen et al., 2005). Simultaneous measurement of the pressure in different nucleus regions is advantageous in indicating the direction and intensity of the internal flow. Improved knowledge on pathways of the fluid movement *via* annulus boundaries and endplates has the potential to shed light on the disc transient response, flow mechanisms, and appropriate *in vitro* conditions when studying disc degeneration, remodeling processes as well as gene and tissue engineering regenerative therapies.

The objective of the present *in vitro* study was hence set to investigate the fluid flow dependent mechanisms in intervertebral discs under repeated loading/ low loading conditions expected in regular daily activities. Since the fluid flow is not easily tractable within the disc, changes in specimen height and pressure at three different nucleus locations were used as surrogate measures to assess the fluid flow within, in and out of the disc. Moreover, to examine the effect of disc initial hydration on its limited recovery capacity post mortem, different preloading and specimen preparation conditions were investigated, evaluated, and discussed.

2. Materials and methods

2.1. Specimen preparation

Fifty four C1–C2 and C2–C3 specimens from skeletally mature bovine tails aged 2–3 years were obtained from a local abattoir and inspected visually to exclude spinal damages. After the removal of all surrounding muscles, soft tissue, facets and transverse processes, each vertebra was sawn approximately 5 mm away from the disc yielding segments consisting of an intervertebral disc with parts of the upper and lower vertebral bodies (Fig. 1a). Both cutting edges were aligned parallel to the corresponding disc mid-plane. They were then wrapped in saline-soaked gauze, placed in plastic bags, and frozen at −20 °C until testing. Twenty hours prior to testing, specimens were thawed at 4 °C in phosphate-buffered saline (PBS, B. Braun Melsungen AG, Melsungen, Germany) to ensure uniform hydrated conditions between specimens. The mean disc diameters (d_{mean}: d_1–d_8), heights (h_{mean}: h_1–h_8) and corresponding areas were measured after initial 18 h thawing period (Table 1).

2.2. Testing devices

Axial compression tests were performed with a servohydraulic material testing machine (858 MiniBionix II, MTS, MN, USA). The force was regulated by a 2500 N load cell and applied in the axial direction with no rotations by a fixed-angle stamp. Specimens were kept in a testing chamber filled with 39 °C (body temperature of cows) PBS (Fig. 2a) allowing thus for a physiologically controlled environment in the entire testing duration. Inside the chamber, specimens were placed between two rigid porous platens allowing a free fluid flow between the bath and the upper and lower surfaces of the vertebral bodies.

2.3. Intradiscal pressure measurements

Three fiberoptic miniature pressure sensors (Ø 0.36 mm, pressure range: −0.1–1.7 MPa) based on the Fabry–Perot principle (360 HP, Samba Sensors, Gothenburg, Sweden) were inserted into each disc; two at the nucleus center at the disc mid-height (NUC) and directly underneath the upper cartilaginous endplate (EP) and one at the nucleus–annulus interface at the disc mid-height (ANN) (Fig. 1). All sensors were inserted *via* an intravascular indwelling cannula, Introcan®, *W*, Ø 0.7 mm (B. Braun Melsungen AG, Melsungen, Germany). The pressure sensors were sutured at the most superficial layers of the disc.

2.4. Loading protocol

To control the degree of disc hydration prior to testing and to allow a reproducible starting point for all tests, the specimens were initially thawed in a bath with PBS for 18 h at 4 °C (Fig. 2b). Subsequently, the specimens were placed in the testing chamber with PBS of 39 °C for additional two hours prior to the beginning of preload compression tests for temperature adjustment.

The loading protocol started with 8 h preload at 0.06 MPa (corresponding to 27.7–45.8 N compressive force depending on the measured disc cross-sectional areas). Subsequently, specimens were subjected to 10 loading/low loading cycles, followed finally by 2 h of low loading at 0.06 MPa. Each loading cycle consisted of 7.5 min axial compression at 0.5 MPa (corresponding to 211.6–373.4 N) and a low loading period of 7.5 min at 0.06 MPa (in the following defined as unloading). To investigate the influence of the preloading, two additional loading regimes were considered. In the first one, the preload magnitude was increased from 0.06 MPa to 0.28 MPa as the average of

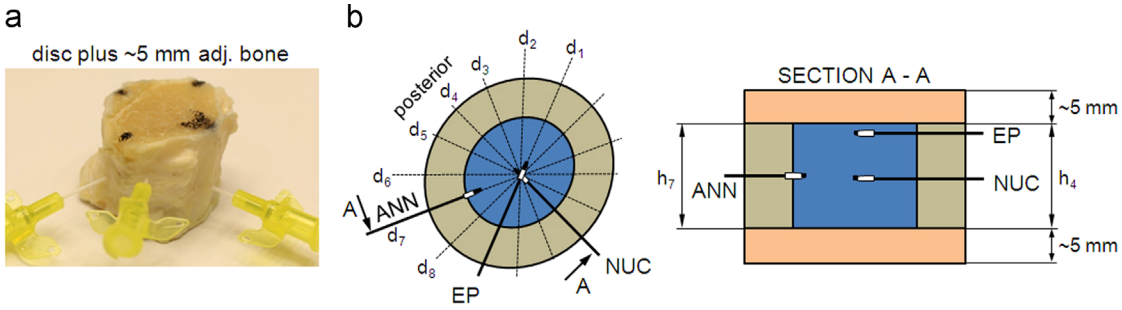

Fig. 1. : (a) Specimen with implanted pressure sensors. Segments consist of an intervertebral disc with approximately 5 mm of adjacent vertebral bodies. (b) Top and section views of the intervertebral disc with inserted pressure sensors: two at the nucleus center at the disc mid-height (NUC) and directly underneath the upper cartilaginous endplate (EP) and one at the nucleus–annulus interface at the disc mid-height (ANN). The arrows on the top view point (A-A) in the directions of viewing for the right figure.