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Long-term culture of bovine nucleus pulposus explants in a native environment

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Abstract

BACKGROUND CONTEXT: Chronic low back pain is a disease with tremendous financial and social implications, and it is often caused by intervertebral disc degeneration. Regenerative therapies for disc repair are promising treatments, but they need to be tested in physiological models.

PURPOSE: To develop a physiological in vitro explant model that incorporates the native environment of the intervertebral disc, for example, hypoxia, low glucose, and high tissue osmolarity.

STUDY DESIGN: Bovine nucleus pulposus (NP) explants were cultured for 42 days in conditions mimicking the native physiological environment. Two different approaches were used to balance the swelling pressure of the NP: raised medium osmolarity or an artificial annulus.

METHODS: Bovine NP explants were either cultured in media with osmolarity balanced at isotonic and hypertonic levels compared with the native tissue or cultured inside a fiber jacket used as an artificial annulus. Oxygen and glucose levels were set at either standard (21% O_2 and 4.5 g/L glucose) or physiological (5% O_2 and 1 g/L glucose) levels. Samples were analyzed at Day 0, 3, and 42 for tissue composition (water, sulfated glycosaminoglycans, DNA, and hydroxyproline contents and fixed charge density), tissue histology, cell viability, and cellular behavior with messenger RNA (mRNA) expression.

RESULTS: Both the hypertonic culture and the artificial annulus approach maintained the tissue matrix composition for 42 days. At Day 3, mRNA expressions of aggrecan, collagen Type I, and collagen Type II in both hypertonic and artificial annulus cultures were not different from Day 0; however, at Day 42, the artificial annulus preserved the mRNA expression closer to Day 0. Gene expressions of matrix metalloprotease 13, tissue inhibitor of matrix metalloprotease 1, and tissue inhibitor of matrix metalloprotease 2 were downregulated under physiological O_2 and glucose levels, whereas the other parameters analyzed were not affected.

CONCLUSIONS: Although the hypertonic culture and the artificial annulus approach are both promising models to test regenerative therapies, the artificial annulus was better able to maintain a cellular behavior closer to the native tissue in longer term cultures.

Keywords: Regenerative therapy; Disc degeneration; Nucleus pulposus; Explant culture; Osmolarity

Introduction

Up to 80% of the population will suffer at least once from low back pain [1,2], and when this disease becomes chronic, its financial and social implications are tremendous. Low back pain can be caused by different mechanisms but is also heavily associated with disc degeneration [3]. During this process, there is a shift from an anabolic to a catabolic environment in the disc; the cells in the nucleus pulposus (NP) decrease their production of the main matrix proteins [4] and increase their production of degrading enzymes [5–7]. As

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a result, the NP loses proteoglycans (PGs) and water [8] and over time changes from a gel-like to a fibrous-like structure [9]. This leads to decreased disc height and loss of function, which ultimately can cause pain [2,10].

Current treatments for low back pain, such as conservative therapy and spinal fusion, do not treat this degeneration and have a limited long-term success. Regeneration of the disc is an alternative long-term approach to treat the disc degeneration at an early stage and prevent low back pain from occurring [11,12]. A number of regenerative therapies have been shown to be promising in animal models [13–16], as they could delay the onset of degeneration [15,16] but still need to be further developed because they could not fully restore the disc to its original healthy state [15–17].

Before these therapies can be used, they need to be tested in a preclinical setting. Animal models (in vivo systems) usually involve induced degeneration by needle aspiration [18] or enzymatic digestion [19], which is different from the human disease [20]. Besides this, they are low-volume throughput, cost intensive, and have ethical considerations [20,21]. Hence, using in vitro models is appealing. Cell culture is less costly and high-volume throughput; however, the native tissue environment is lost during cell isolation, which might affect the cellular behavior to the regenerative stimuli. From all in vitro models, whole-disc explants preserve the native environment best but their throughput volume is still low and their stability in the long-term culture is limited [22]. Nucleus pulposus explant culture is another suitable model because the earliest detectable changes during degeneration occur in the NP [23] and the native tissue environment can be maintained [24] in such a model.

Previously we have successfully cultured bovine NP explants for 21 days [25]. In that study we used polyethylene glycol (PEG) to raise the medium osmolarity to the native level and showed that we were able to prevent the swelling and PG loss. However, the cellular behavior was different from the fresh tissue at the gene level. We hypothesized that although balancing the osmolarity is important to prevent PG loss at the tissue level, other factors in the native environment (eg, hypoxia, low glucose levels, and low pH [26]) might be important for the cellular behavior. Indeed, it has been shown that glycosaminoglycan (GAG) production is maximal at 5% O_2 for NP explants cultured for 24 hours [27] and also at a pH of 7.1 when cultured for 4 hours [28]. Therefore, it appears important to culture NP explants under physiological O_2, glucose, and pH [29].

A second possible explanation for the difference in the cellular behavior we previously observed is that a balanced medium osmolarity is different from the physiological situation in which the annulus fibrosus contains NP swelling. Our second hypothesis is that an artificial annulus approach is more physiological than a balanced medium osmolarity approach and might benefit the cellular behavior in NP explants.

To see the effects of regenerative therapies on the tissue level, we need a model that is stable for even longer than 21 days of culture; therefore, the culture duration was extended to 42 days. Furthermore, we analyzed the explants at Day 3 to assess if the changes in the cellular behavior were a direct effect of the harvesting and culture initiation stress or occurred over time in culture.

The aim of this study was to improve our NP explant model by incorporating more factors of the native physiological environment and to determine the importance of the separate factors. Therefore, we cultured NP explants for 6 weeks under physiological O_2 and glucose levels in a swelling balanced environment using either an osmotic balance or an artificial annulus approach. We analyzed the tissue composition with biochemical assays and histology and the cellular behavior with gene expression.

Materials and methods

Tissue sampling

NP explants were harvested from fresh caudal discs of 24-month-old cows. These were obtained from the abattoir according to the local regulations, and a total of 70 discs (CC2–CC5) were harvested from 18 donors. The different levels were distributed equally and randomly among the different conditions. The discs were opened transversally directly underneath the end plate, and NP explants were punched out with an 8-mm diameter biopsy punch (Kruuse, Sherburn, UK) from the center of the NP.

Medium

Standard medium was prepared from basic Dulbecco's Modified Eagle Medium powder (Gibco; Invitrogen, Carlsbad, CA, USA) in milli-Q filtered water (8.3 g/L), supplemented with 15.9 mg/L phenol red (Sigma, Zwijndrecht, The Netherlands), 2% L-glutamine (Lonza, Basel, Switzerland), 1% pyruvate (Gibco), 1% penicillin/streptomycin (Lonza), 3.7 g/L sodium bicarbonate (Sigma), 50 mg/L ascorbic acid (Sigma), and 10% fetal bovine serum (Gibco). Glucose was added to the media for a final concentration of 4.5 g/L for the standard conditions or 1 g/L for the physiological conditions. All media were filter sterilized and the pH was adjusted to 7.1, the pH of a healthy human disc [30].

PEG culture

In PEG culture, the medium osmolarity was adjusted to two levels based on the previous study [25]

- Isotonic to native in situ NP (430 mOsm/kg H_2O): standard medium+8.2% w/v PEG (20kD, Sigma)
- Hypertonic to native in situ NP (570 mOsm/kg H_2O): standard medium+13.3% w/v PEG.

NP explants were placed inside the dialysis tubing (15 kD molecular weight cut-off, Spectra-Por, Rancho Dominguez,