Osmotic Loading Effects on Juvenile Intervertebral Disc Cell Biosynthesis is Dependent on Cell Type and TGF-β_3

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Introduction

Approximately 25% of the disc's fluid is expelled and re-imbibed during each diurnal cycle. The decrease in water content significantly alters the osmotic environment from 400mOsm at maximum hydration to 550mOsm after maximum daily fluid expulsion [1, 2]. Disc degeneration is noted by a decrease in the water content and a loss in full recovery from diurnal loading, which may further increase the internal osmotic loading environment of the disc [3, 4]. Short duration studies (i.e. 5 days) have demonstrated that NP cell synthesis of aggrecan increases by 40-100% when cultured in culture medium that mimics the in vivo osmotic environment (i.e. 400-500mOsm) [2, 5]. A recent study using chondrocytes from articular cartilage demonstrated that hyperosmotic loading in pellet culture increased protein production (GAG and collagen) [6]. The objective of this study was to determine the effect of long-term osmotic loading on matrix turnover and proliferation by disc cells.

Methods

Lumbar spine sections from juvenile cows (3-6 weeks) were acquired and the disc was removed aseptically with a scalpel. The disc was then further divided into three regions, including the AF, NP, and EP (Fig 1). The tissues were digested in serum media containing collagenase. Micro-pellets were formed from passaged cells for each cell type by centrifugation and cultured in chemically-defined media supplemented with TGF-β_3 [6]. The osmolarity of the culture media was diluted to make 300mOsm media by adding deionized, distilled water (starting osmolarity ~334mOsm). Then, NaCl and KCl were added to the media to make 400mOsm and 500mOsm media. At day 14, each osmolarity group was divided into two groups, one half of the pellets continued to receive growth factor supplement and the other half was 'released' from the growth factor supplement. Biochemical analysis was performed for DNA, GAG and collagen on days 0, 14 and 28 (n = 5 per group per time point). The GAG content was measured in the pellet and the culture media. The doubling rate was calculated at day 14 as $T_d = 14*\log(2)/\log(DNA_{14}/DNA_0)$ for cells cultured in 300 mOsm. A one-way ANOVA was performed with a Bonferroni's post hoc test to determine differences in the biochemical content of pellets cultured with continuous growth factor supplementation. At day 28, a t-test was performed to compare the biochemical content of pellets cultured in transient and continuous growth factor supplementation. Significance was set at α=0.05.

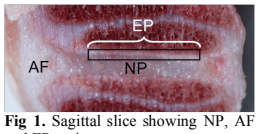

Fig 1. Sagittal slice showing NP, AF and EP regions.

Results

The doubling rate for AF and EP cells (26 and 33 days, respectively) was greater than the doubling rate for NP cells (50 days). Hyperosmotic (400 & 500mOsm) loading decreased cell proliferation for all cell types (Fig 2- asterisk). There was a parabolic response in the GAG production with osmotic loading, where pellets cultured in 400 mOsm media producing more GAG than pellets in the 300 mOsm or 500 mOsm media (Fig 3- †). Pellets in the hyperosmotic loading conditions released more GAG into the media than the 300mOsm group; therefore, there was no difference in the GAG content normalized by DNA (p>0.05). The GAG content normalized by the DNA for the 400 mOsm group was 37.8 ± 4.9 g/g for the NP, 31.4 ± 2.4g/g for the AF, and 69.9 ± 12.8g/g for the EP (p<0.01 for EP vs. NP and AF). At day 28, there was a trend for hyperosmotic loading to increase collagen production in NP pellets (p=0.06) and decrease collagen production in AF pellets (p=0.08; Fig 4).

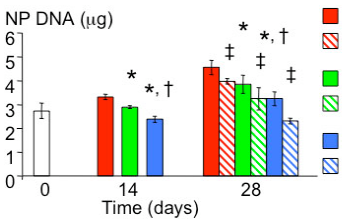

Fig 2. NP DNA content for pellets cultured in continuous (C) and release (R) growth factor conditions. *p≤0.05 vs. 300C; †p≤0.05 vs. 400C; ‡p≤0.05 C vs. R.

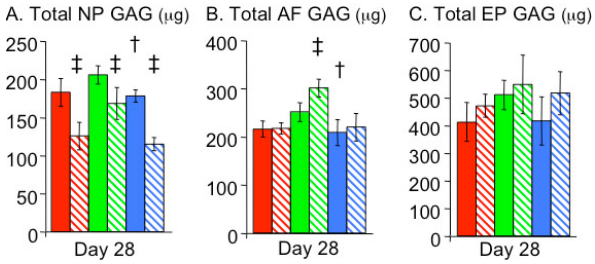

Fig 3. Total GAG content calculated as the GAG in the media and the GAG in the pellet for A) NP, B) AF and C) EP cells. †p ≤0.05 vs. 400C; ‡p≤0.05 C vs. R. See Fig 2 for the legend.

There was no effect with the collagen content in EP pellets with osmotic loading (day 28 = 15.7 ± 2.5 g/g; p=0.2).

In the NP pellets, transient application of growth factors ('release' group) decreased cell proliferation and GAG production (Fig 2 & 3 - ‡), but did not alter collagen production. Generally, matrix production and cell proliferation of the AF and EP pellets was not affected by the transient application of growth factor.

Discussion

As future treatments for painful degenerated discs move towards a biological replacement or repair, it is important to understand the effect the in situ environment will have on tissue maintenance, cell production and proliferation. GAG results of this study suggest that 400 mOsm may be the optimal environment for matrix production by juvenile bovine disc cells, and support the use of hyperosmotic loading as a means for tailoring the disc cell environment, in a biomimetic approach, for IVD tissue engineering and repair applications.

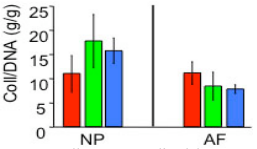

Fig 4. Collagen normalized by DNA for NP and AF (p≤0.08). See Fig 2 for the legend.

Interestingly, the disc cells responded differently to transient or continuous supplementation of growth factors. GAG production was higher for NP cells cultured with continuous growth factor supplementation, which is consistent with observations with adult chondrocytes [7]. In contrast, the AF and EP cells had higher matrix synthesis with transient growth factor, which is consistent with observations with juvenile chondrocytes [8]. More work is needed to understand the differential effect of growth factors on these disc cell populations.

The juvenile disc model was adopted in the current study as it provides a robust system to study the influence of environmental cues on disc cell biosynthesis. The GAG/DNA ratio was highest for the EP cells, as expected, due to the cartilaginous environment *in situ*. However, the GAG/DNA ratio for AF cells was comparable to the GAG/DNA ratio of NP cells, which may suggest that AF cells could be a potential cell source for future treatments of degenerated NP tissue. As some differences exist between the matrix turnover measured on juvenile bovine cells in this study and previous work examining osmolarity effects on gene expression of mature disc cells [9], future work adopting the experimental design described herein will be expanded to include adult disc cells.

Significance

Fluid is expelled during diurnal loading and the water content of the disc decreases with degeneration, which likely increases the osmotic environment of the tissue. Hyperosmotic loading decreases cell proliferation of disc cells in the endplate (EP) annulus fibrosus (AF) and the nucleus pulposus (NP). Glycosaminoglycan (GAG) production for all cell types was highest at for osmotic loading conditions near native values (400mOsm).

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References [1] Boos+ *Radiology* 1993; [2] Sivan+ *Biorheology* 2006; [3] O'Connell+ *JMBBM* 2011; [4] Antoniou+ *J Clin Invest* 1996; [5] Ishihara+ *Am J Physiol* 1997; [6] Reza+ *Biotech & Bioeng* 2010; [7] Bian+ *Tissue Eng* 2009; [8] Huang+ *Tissue Eng* 2009; [9] Neidlinger-Wilke+ *JOR* 2011.