Maturation and Material Dependent Response of AF and NP Cells to Mechanical Perturbation

[1]Dong Hwa Kim, PhD
[1,2,3]Lachlan J. Smith, PhD
[4]Dawn M. Elliott, PhD
[1]Robert L. Mauck, PhD

[1]University of Pennsylvania, Philadelphia, PA, USA

[2]Translational Musculoskeletal Research Center, Philadelphia VA Medical Center, Philadelphia, PA, USA

[3]Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA, USA

[4]University of Delaware, Newark, DE, USA

Introduction

The mechanical environment of the intervertebral disc (IVD) plays an important role in regulation of extracellular matrix (ECM) biosynthesis and maintenance of the unique disc cell phenotypes. The adult IVD comprises cell populations of two distinct lineages (nucleus pulposus cells (NPCs) and annulus fibrosus cells (AFCs)) that are segregated into different functional compartments in the disc, and exposed to different loading environments.[1] NPCs, which reside in a proteoglycan-rich hydrated NP extracellular matrix, are exposed to compressive and hydrostatic pressure. AFCs, which reside within a dense organized fibrous AF network, experience tensile deformation.[2,3] Because cells can rapidly lose their phenotypic characteristics when isolated from tissue, in vitro culture in an appropriate 3D physical environment is crucial. For instance, encapsulating NPCs in hydrogels and culturing AFCs on aligned nanofibrous scaffolds provides a 3D physical environment that is consistent with the native tissue architecture, while also promoting retention of phenotype and organized matrix formation with in vitro culture.[4] The objective of the current study was to investigate the response of NPCs and AFCs subjected to short term physiologically-relevant mechanical stimulation in 3D culture systems appropriate to the cell phenotype (i.e., compressive loading in hydrogel culture for NPCs and cyclic tensile strain in nanofibrous scaffolds for AFCs), and to determine whether the response to mechanical loading would change as a function of culture duration.

Methods

NPCs and AFCs were isolated from adult bovine caudal discs.[5,6] NPCs (passage 2) were encapsulated in 1% methacrylated hyaluronic acid (HA) at 60×10^6 cells/ml and photopolymerized into cylindrical constructs (diameter and thickness, 4 mm and 2.25 mm, respectively).[7] AFCs (passage 2) were seeded onto aligned poly(ε-caprolactone) nanofibrous scaffolds at 2×10^6 cells/scaffold.[8] Constructs of both types were cultured in a chemically defined medium [CM(−)] for two days, after which they were subjected to mechanical loading. Another set of constructs was cultured in chemically defined media supplemented with TGF-β3 [CM(+)] for 42 days, followed by transfer to CM(−) for 5 days, before being subjected to the same loading protocol. At both time points, NPC-seeded constructs were loaded in dynamic compression (2% pre-strain, 10% strain, 1 Hz),[9] while AFC-seeded constructs were subjected to cyclic tensile strain (6%, 1Hz) for 4 hours.[9] Construct mechanical properties for each condition were determined via unconfined compressive stress relaxation and tensile testing of NP and AF constructs, respectively, as previously described.[8,9] The equilibrium modulus of NP constructs was determined from the equilibrium stress and strain values normalized to construct dimensions. The tensile modulus of AF constructs was determined from the linear region of the stress-strain curve (1%/sec extension), based on the sample geometry and gauge length. Cell viability was assessed via Live/Dead staining (Molecular Probes) and routine histological staining of matrix (collagen and proteoglycan) was performed. Real time RT-PCR was used to analyze expression of matrix proteins [aggrecan (ACAN), collagen type I (COL1A1), collagen type II (COL2A1)], SOX9, and TGF-β normalized to GAPDH. Statistical differences were established by t-tests or ANOVA with Fisher's LSD post-hoc.

Results

Both NPC-seeded and AFC-seeded constructs increased in mechanical properties with time in culture. By day 42, the equilibrium modulus of NPC-seeded HA constructs was 4-fold greater than at day 2 (Figure 1A, $p<0.05$). Similarly, the tensile modulus of AFC-seeded nanofibrous scaffolds was 1.8-fold greater for day 42 constructs compared to day 2 constructs (Figure 1B). Cell viability in both NPC-seeded and AFC-seeded constructs was high at both time points (not shown), and abundant extracellular matrix was present by day 42 (not shown). NPCs were generally rounded/stellate in HA gels, while AFCs were highly aligned with the nanofiber direction. When NPC-seeded constructs were subjected to mechanical perturbation, ACAN

Poster No: 1617
2014 Annual Meeting
Orthopaedic Research Society
kimd1@mail.med.upenn.edu

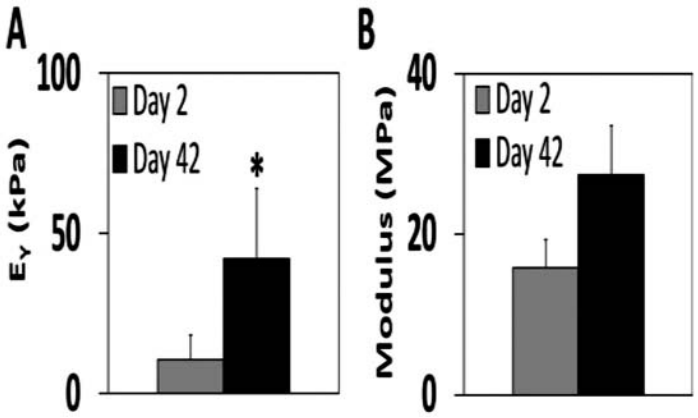

Figure 1. (A) Equilibrium compressive modulus of NPC-seeded HA constructs and (B) tensile modulus of AFC-seeded nanofibrous scaffolds over 42 days of in vitro culture (*: $p < 0.05$ vs. Day 2, n=4~5/group/time point).

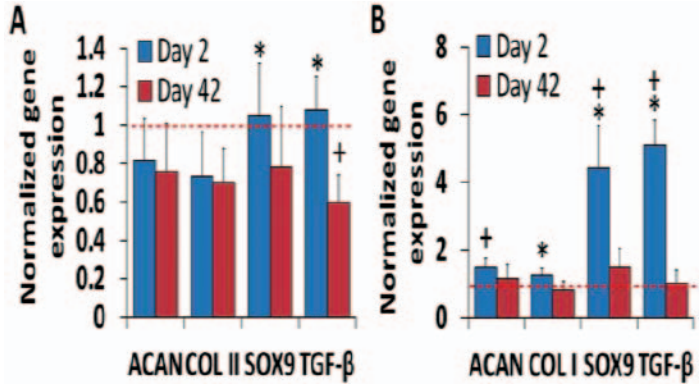

Figure 2. (A) Gene expression in NP constructs with compressive loading (dashed line: free swelling condition, n=4~5, *: $p<0.05$ vs. Day 42, +: $p<0.05$ vs. free swelling). (B) Gene expression in AF constructs with tensile loading (dashed line: free swelling condition, *: $p<0.05$ vs. Day 42, +: $p<0.05$ vs. free swelling).

and COL2A1 expression decreased at early time points, while SOX9 and TGF-β expression did not change (Figure 2A). At later time points, compressive stimulation resulted in a decrease in expression levels of all genes assayed, with SOX9 and TGF-β expression levels at this later time point were significantly lower than at the early time point (Figure 2A). In AFC-seeded constructs, dynamic tensile loading at the early time point significantly increased ACAN, SOX9 and TGF-β gene expression. Conversely, after these constructs had matured through day 42, dynamic stretch did not alter expression of any gene assayed (Figure 2B).

Discussion

In this study, we investigated the effect of physiologic mechanical loading on AF and NP cells cultured 3D physical environments that promote their respective phenotypes, at both early and late time points. Gene expression of ECM and signaling molecules differed based on material context and duration of culture before mechanical perturbation. In NPC-seeded constructs, compressive loading generally inhibited gene expression, particularly after constructs had been matured for longer durations. Conversely, dynamic tensile loading of AFC constructs markedly increased expression of select genes early in culture, but had a lesser effect at later time points. These data suggest that as matrix is deposited, the manner in which cells within these materials interpret physical signals changes, most likely due to cell-mediated matrix deposition. Future studies will explore how these responses are modulated by culture duration and ECM deposition, and how these cell types signal to one another with physiologically relevant mechanical perturbation.

Significance

The mechanical environment of the IVD is complex, with different regions of this composite tissue exposed to different loading configurations. In this study, we investigated the effect of physiologically relevant mechanical stimulation of NPC and AFC seeded in 3D culture systems that promote their phenotypic stability. Findings show that duration of maturation alters the response of these cells to physical signals.

Acknowledgments

Funded by the DOD (#OR090090) and the Department of Veterans Affairs, with support from the Penn Center for Musculoskeletal Disorders.

References

1. Urban JPG. The role of the physicochemical environment in determining disc cell behaviour. *Biochem. Soc. Trans.* 30, 858–864 (2002).

2. McNally DS, Adams MA. Internal intervertebral disc mechanics as revealed by stress profilometry. *Spine* 17, 66–73 (1992).

3. Stokes IA. Surface strain on human intervertebral discs. *J. Orthop. Res.* 5, 348–355 (1987).

4. Nerurkar NL, Elliott DM, Mauck RL. Mechanics of oriented electrospun nanofibrous scaffolds for annulus fibrosus tissue engineering. *J. Orthop. Res.* 25, 1018–1028 (2007).

5. Delaine-Smith RM, MacNeil S, Reilly GC. Matrix production and collagen structure are enhanced in two types of osteogenic progenitor cells by a simple fluid shear stress stimulus. *Eur. Cell. Mater.* 24, 162–174 (2012).

6. Nerurkar NL, et al. Nanofibrous biologic laminates replicate the form and function of the annulus fibrosus. *Nat. Mater.* 8, 986–992 (2009).

7. Kim et al. BMES 2013.

8. Huang AH, Farrell MJ, Mauck RL. Mechanics and mechanobiology of mesenchymal stem cell-based engineered cartilage. *J. Biomech.* 43, 128–136 (2010).

9. Baker BM, Shah RP, Huang AH, et al. Dynamic tensile loading improves the functional properties of mesenchymal stem cell-laden nanofiber-based fibrocartilage. *Tissue Eng. Part A* 17, 1445–1455 (2011).