TGF-b Improves Cell Viability in Human-Sized Disc-Like Angle Ply Structures (DAPS)

Dong Hwa Kim, PhD[1]
Sarah Gullbrand, PhD[1]
Dawn Elliott, PhD[2]
Lachlan Smith, PhD[1]
Harvey Smith, MD[1]
Robert Mauck, PhD[1]

[1]University of Pennsylvania and Philadelphia VA Medical Center Philadelphia, PA

[2]University of Delaware, Newark, DE

Introduction

A number of whole-disc tissue engineering strategies have emerged for the replacement of pathologic discs[1,2], with some recent studies showing promise *in vivo*[3,4]. Our group recently developed disc-like angle ply structures (DAPS) sized for the rat tail (small, 1.5 mm height, 4 mm diameter) and showed that these constructs achieved near native composition[3, 5]. However, a major challenge remains in scaling these constructs to human size, where homogeneous matrix deposition will be required throughout a large expanse[6]. Transforming growth factor-beta (TGF-β) is one of the most widely utilized mediators for tissue engineering, as it can spur matrix formation[7]. However, our findings and those of others have shown that supplementation with active TGF-β results in heterogeneous matrix accumulation, concentrated near the periphery. To overcome this limitation, one recent publication described the provision of latent TGF-β alongside active TGF-β, and showed that this enhanced cell and matrix distribution in chondrocyte- based cartilage constructs[8]. In the present study, beginning with a chemically defined medium[5], we assessed the impact of latent TGF-β supplementation on nucleus pulposus (NP) cell matrix production and distribution in a 3D-hydrogel and DAPS culture system sized for human application.

Methods

NP cell-laden agarose hydrogel culture

NP cells were encapsulated in a 2% agarose hydrogel at a density of 20 million cells/mL. Constructs (diameter: 10 mm, thickness: 4 mm) were cultured for 5 weeks in one of three media conditions: chemically defined media (CDM) with 10 ng/ml TGF-β3 (Active TGF), CDM with 43 ng/ml latent TGF-β1 (Latent TGF), or Active + Latent TGF media (Active + Latent TGF).

Large sized DAPS fabrication and culture

Large sized DAPS (6 mm × 20 mm outer diameter, NP diameter = 10 mm) were next fabricated. Electrospun poly(ε-caprolactone) (PCL) aligned sheets (thickness: 250-300 μm) were used for developing the AF region of the DAPS by cutting sheets into strips at a 30 degree angle[3]. Bovine AF cells (3,333 cells/mm^2) were seeded onto the strips and cultured for 1 week in CDM containing active TGF-β. To form the circular AF region, with alternating fiber orientations in each layer, strips were coupled and wrapped concentrically using a custom mold[5]. To fabricate the NP region of the DAPS, bovine NP cells were encapsulated in 2% agarose (20 million cells/mL) and cultured for 2 weeks in chemically defined media containing active TGF alone, or active + latent TGF prior to combining with the AF region.

Viability, mechanical properties, histological assessment, and quantitative T2 MRI

Construct halves were stained with Live/Dead for cell viability. A custom MATLAB program was used to automate counting of cells in each region. Compression testing was carried out as in[5]. Additional samples were stained with Alcian blue and picrosirius red to visualize glycosaminoglycans (GAG) and collagen, respectively. Structure and NP hydration were also assessed by quantitative T2 MRI, as in[5].

Results

Regional assessment of viability at 5 weeks showed a depth-dependent decline in viability in Active and Latent TGF-supplemented NP agarose constructs, especially in the central region. In contrast, simultaneous Active + Latent TGF supplementation resulted in a more homogeneous distribution of living cells (Figure 1A). Histology also showed intense staining for GAGs though the tissue sections in this Active + Latent TGF supplemented construct (Figure. 1B). Next, large sized DAPS were successfully fabricated and cultured in media containing Active TGF with/or without Latent TGF for up to 10 weeks (Figure 2A). Active + Latent TGF-supplemented DAPS had a significantly higher cell viability in the center of NP region compared to those cultured in Active TGF alone (Figure 2B, C). The transition strain also significantly decreased in the Active + Latent TGF-supplemented DAPS at 10 weeks. However, there were no significant differences in the toe region modulus between Active TGF and Active + Latent TGF DAPS at this time point (Figure 2D). For both groups, histology showed intense staining for GAGs and collagen at the tissue periphery, but far less collagen staining in the

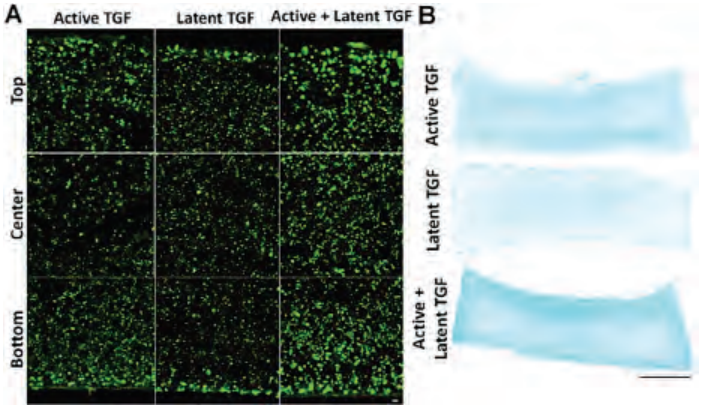

Figure 1. (A) Representative Live/Dead staining of NP cell-laden agarose gels cultured in three different media formulations for 5 weeks (Bar = 10 μm). **(B)** Alcian Blue staining of proteoglycans (Bar = 2mm).

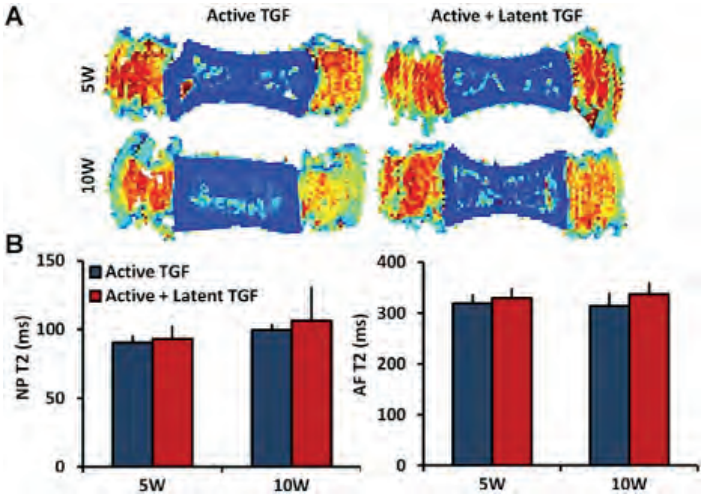

Figure 3. (A) Composite T2 maps for each experimental group. **(B)** NP and AF T2 values over 10 weeks culture (n = 3).

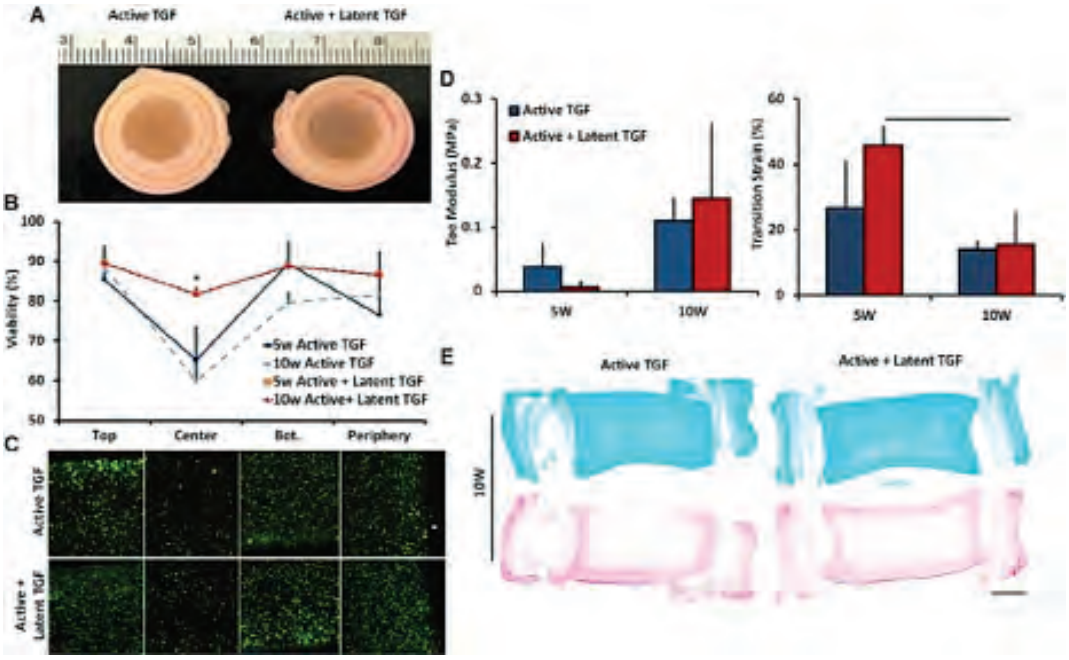

Figure 2. (A) Gross appearance of DAPS cultured for 10 weeks. **(B)** Quantification of viability (n = 3, *:$p < 0.05$ vs. 5w and 10w Active TGF) **(C)** Live/Dead viability staining. **(D)** Mechanical properties of DAPS (n = 3) E. Alcian blue (top) and picrosirius red (bottom) stained DAPS at 10 weeks (scale = 2 mm).

tissue interior (Figure 2E). NP and AF T2 values of Active + Latent TGF-supplemented DAPS were similar to that of Active TGF alone cultured DAPS (Figure 3A,B).

Discussion

This study explored the impact of Active TGF and Latent TGF supplementation on the homogeneous growth and maturation of large sized DAPS in vitro. Our findings demonstrate that combination of Active TGF with Latent TGF dramatically improves disc-like ECM content with homogeneous cell viability in these large NP cell-laden hydrogel constructs. Notably, despite the increased cellularity, Active TGF mixed with Latent TGF had no impact on construct properties. Furthermore, we noted persistent heterogeneity in matrix distribution under these conditions. These findings suggest that while provision of Latent TGF can improve cell viability in large constructs, additional work is required to optimize matrix deposition when the NP is coupled to an AF region.

Significance

Our findings suggest that the combination of Active TGF and Latent TGF can mitigate gradients in viability observed in large tissue constructs. Further optimization of this growth is needed for improving the human translation of tissue engineered discs.

References

1. **Mizuno**+ 2004 *Spine.*
2. **Mizuno**+ 2006 *Biomaterials.*
3. **Martin**+2014 *Acta Biomat.*
4. **Bowles**+ 2011 *PNAS.*
5. **Gullbrand**+ 2017 *ISSLS proceedings.*
6. **Bian**+ 2009 *Osteoarthr. Cartilage.*
7. **Richardson**+ 2006 *Stem Cells.*
8. **Albro**+ 2016 *Biomaterials.*