Differential Effects of Growth Factors on Neonatal and Adult Achilles Tenocytes

J Huegel
Robert L. Mauck
Louis J. Soslowsky
Andrew F. Kuntz

McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

Introduction

Tendon tears in adults are a common orthopaedic problem and often fail to heal even after surgical repair. Neonatal healing is superior to adult healing across multiple tissue types,[1] and immunohistochemical studies in tendons have shown age-related differences in matrix composition and growth factor expression.[2-3] However, it is not known whether adult cells have an innate inability to synthesize matrix and initiate a successful repair, or whether the adult wound environment lacks sufficient presentation of growth factor stimuli to affect repair. During the repair process, several growth factors act as cues to initiate and regulate cellular responses such as cell proliferation and extracellular matrix production. Therefore, we hypothesized that neonatal tendon cells would be more responsive than adult cells to two growth factors that are upregulated during tendon healing:[4] transforming growth factor β3 (TGFβ3) and basic fibroblast growth factor (bFGF).

Methods

Achilles tendons were harvested from postnatal day 3 (P3, neonatal) and five month old (adult) male Sprague-Dawley rats. Tendons were finely diced and placed in culture dishes with Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells migrated out of the tissue and were expanded to confluence. To evaluate age and growth factor effects on tenocyte migration, a transwell assay (8 micron, Millipore) was performed with 3 concentrations[5-6] of TGFβ3 and bFGF. Cells were plated at 2×10^5 cells/ml and incubated for 6 hours with the specified factor in serum-free DMEM. Migration towards growth factor supplemented media was normalized to cell migration towards serum-free DMEM. To evaluate proliferative effects of growth factors, cells were plated at 5000 cells per well in a 96 well plate and treated with either TGFβ3 or bFGF in phenol-free DMEM supplemented with 2.5% FBS. MTT assays (Life Technologies) were performed on days 3 and 6. To quantify the effect of growth factors on collagen production, a Sircol collagen assay (Biocolor) was performed. Cells were plated in 12 well dishes at 1×10^5 cells per well and treated with TGFβ3 or bFGF in DMEM supplemented with 2.5% FBS. After 6 days, aliquots of media were complexed with Sircol dye reagent and analyzed colorimetrically. 2-way ANOVA with repeated measures with follow-up t-tests between groups at each time point were performed with statistical significance set at $p < 0.05$ and trends at $p < 0.1$.

Results

Tenocyte Proliferation: After three days, all concentrations of growth factors induced a significant increase in proliferation in adult cells, whereas neonatal cells only trended towards greater proliferation (Figure 1). After six days, adult cells continued to respond to bFGF but not to TGFβ3 (Figure 1). Higher concentrations of both bFGF and TGFβ3 induced significantly more neonatal cell proliferation than control cultures.

Tenocyte Migration: At 6 hours, all concentrations of bFGF and TGFβ3 induced greater migration than serum-free media (Figure 2). Similar to the proliferative response, a greater number of neonatal cells migrated towards 10% FBS supplemented media than adult cells. *Collagen production:* In adult cells, TGFβ3 increased collagen production compared to basal media (Figure 3). In contrast, neonatal cells were more responsive to bFGF. Most interestingly, neonatal cells produced greater than two-fold more collagen than adult cells at baseline conditions (2.5% FBS in DMEM). When treated with 10% FBS, both types of cells produced a quantity of collagen above the detectable range of the assay.

Discussion

During tendon healing, growth factor cues stimulate cell proliferation and migration to the site of injury, as well as collagen deposition and reorganization. Our data show that both neonatal and adult tenocytes respond to exogenous growth factors, though the pattern of response differs based on cell age. Adult cells proliferated and migrated similarly to neonatal cells in response to growth factors, suggesting that these cells are capable of responding to stimuli to initiate a healing response. Along with previously reported differences in growth factor

Correspondence:
Louis J. Soslowsky
McKay Orthopaedic Laboratory, University of Pennsylvania, Philadelphia, PA, USA
soslowsk@upenn.edu

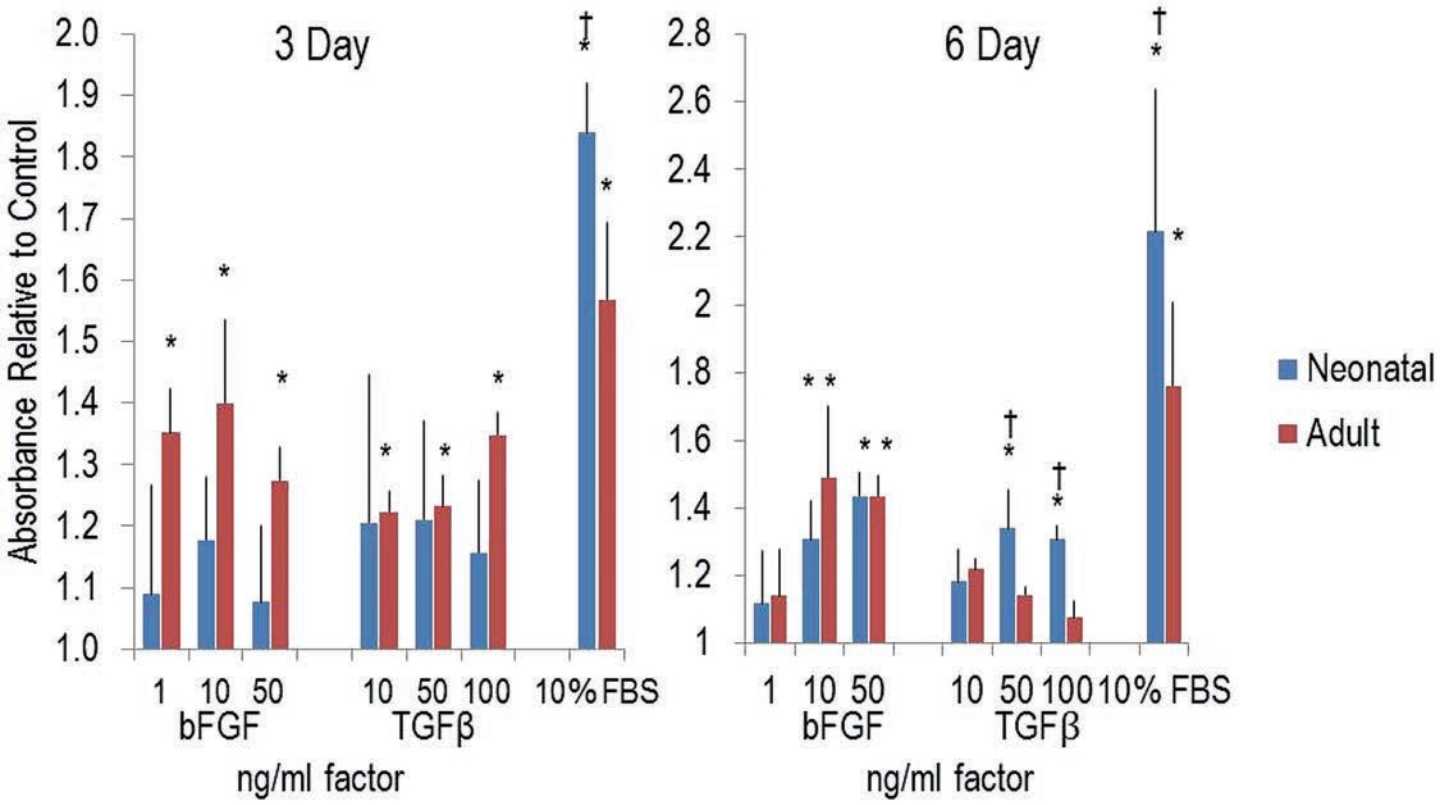

Figure 1. MTT proliferation after 3 and 6 days of growth factor treatment. bFGF and TGFβ induce a mitogenic response early in adult cells, but the response is only sustained by bFGF treatment. Neonatal cells are slower to respond. Asterisks indicate significant differences from control treatments ($p < 0.05$). Crosses indicate significant differences between cell types ($p < 0.05$).

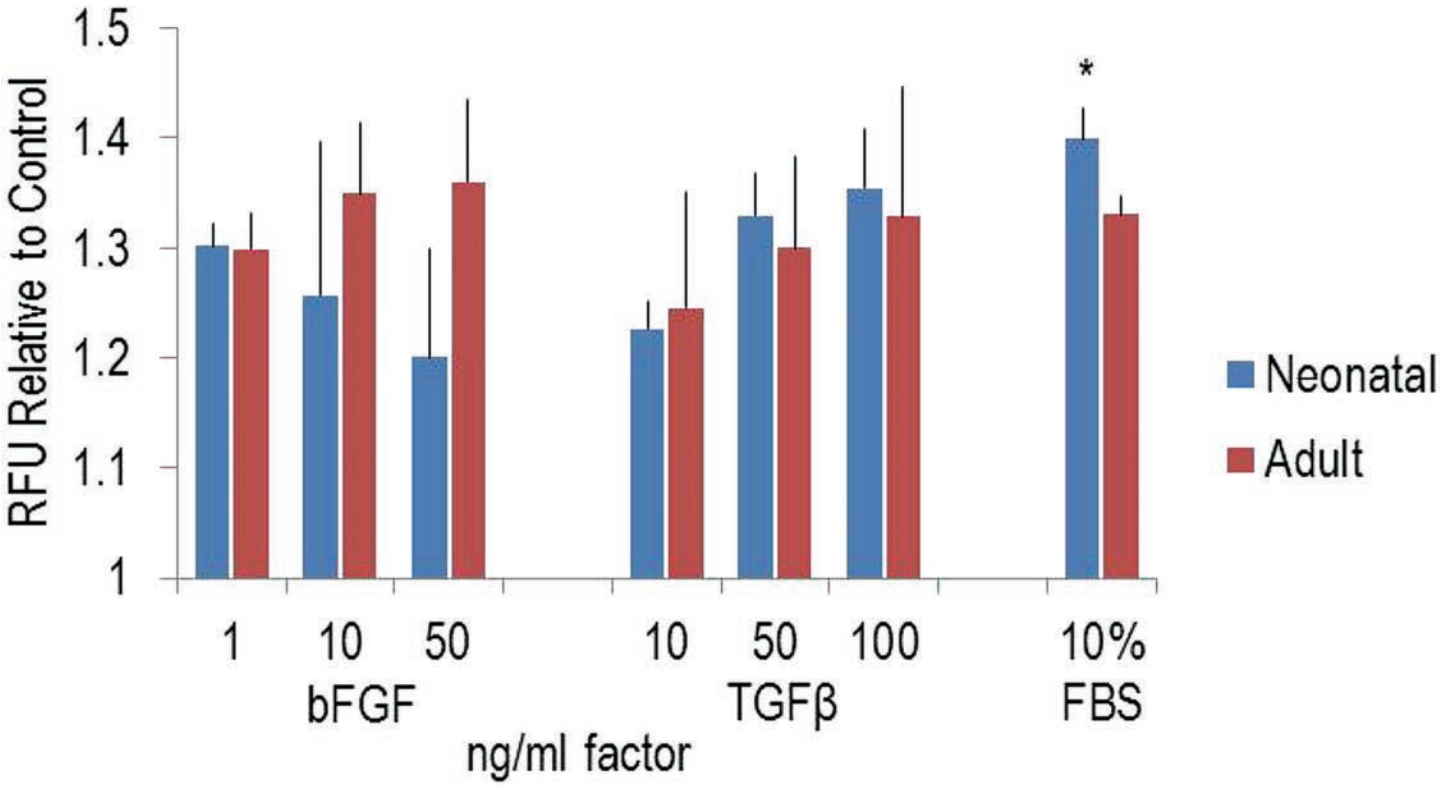

Figure 2. Transwell migration assay. bFGF and TGFβ act as chemotactic agents for every concentration of both factors, with no significant differences between cell types. Asterisks indicate significant differences from control treatments ($p < 0.05$).

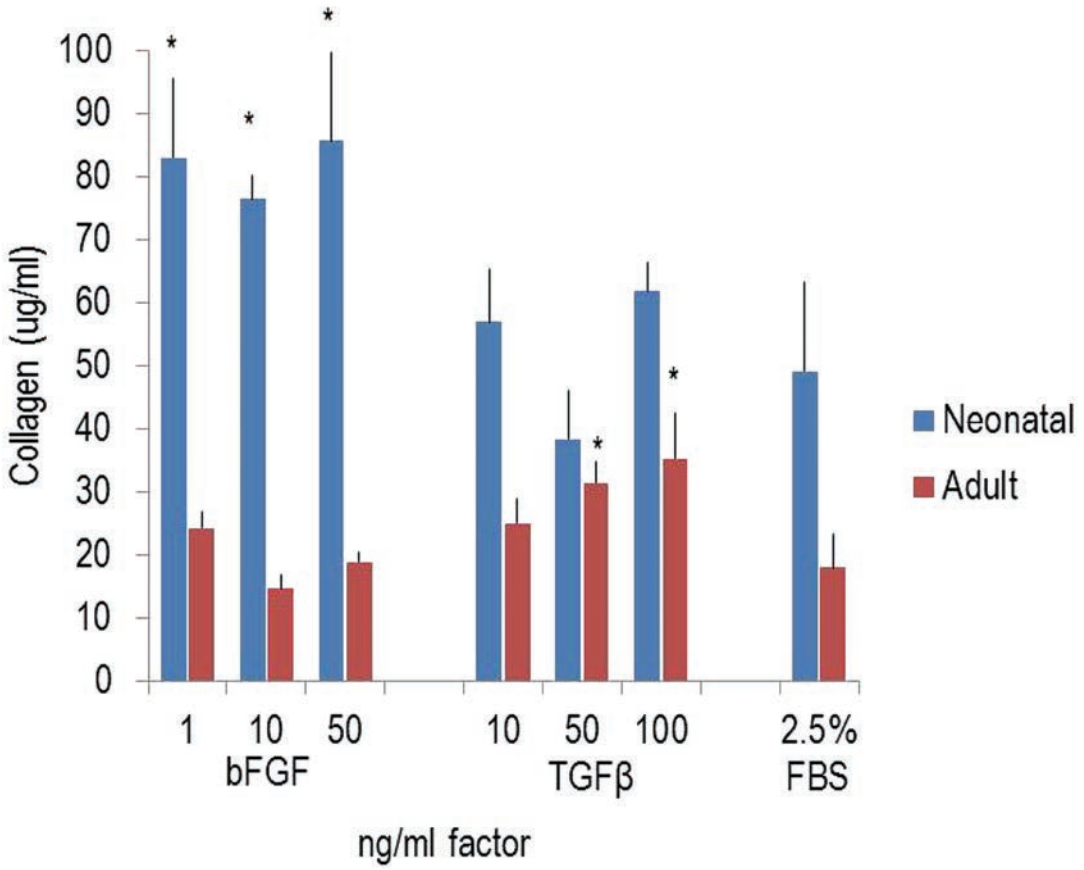

Figure 3. Sircol collagen quantification assay. bFGF induced collagen production in neonatal cells while adult cells were more responsive to TGFβ. Overall, neonatal cells responded more significantly to all treatments including basal media. Asterisks indicate significant differences from control treatments ($p < 0.05$). All neonatal groups were significantly higher than adult groups.

expression during aging, the data support the concept that these cells may simply lack growth factor stimuli in their environment. Tissue engineered approaches to tendon healing often include the addition of a growth factor to enhance healing. This *in vitro* data supports the value of growth factor treatments in adult tendons.

While some commonalities in response were noted, major differences between neonatal and adult cells were also delineated. First, neonatal cells responded more robustly to FBS than adult cells. FBS contains a combination of factors including bFGF and TGFβ as well as other factors known to enhance tendon healing such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). This combination of factors may be more representative of a biological healing response, where a combination of factors may yield an overall synergistic effect. The increased response in neonatal cells suggests that younger cells have a greater capacity to respond to such a combination of factors than adult cells. Indeed, results suggest that neonatal cells are capable of producing greater than five times more collagen than adult cells over the same period of time. Because the production and reorganization of collagen is vital to the regeneration of such a load-bearing tissue, this result may be the primary distinction between young and old tissue healing. However, the addition of the highest concentration of TGFβ did increase collagen production in adult cells by two-fold over control treatment, suggesting that stimulation of collagen production in older tissue is feasible. Future studies will establish the type and quality of collagen production after *in vivo* delivery of growth factors to injured tendons in order to determine the optimal dose and delivery timeline.

Significance

The poor intrinsic healing capacity of adults predisposes surgical repair of tendons to potential failure, while fetal and neonatal tissues provide a more robust healing response and can achieve mechanical properties closer to that of uninjured tissue. This study demonstrated that adult tenocytes respond similarly to neonatal cells in some aspects of the healing response, particularly in the response to pro-mitotic and pro-migratory factors, though neonatal cells showed an overall higher level of collagen biosynthesis. More broadly, this study supports the targeted use of exogenous growth factors in tissue-engineered tendon repair approaches in adults.

Acknowledgements

This study was supported by the University of Pennsylvania Institute on Aging. We thank Jennica Tucker and Ben Freedman for dissection assistance.

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