Effects of Autologous Tenocyte-Seeded Nanofibrous Scaffolds in Rotator Cuff Repair are Age-Dependent

Huegel J[1]
Kim DH[1]
Cirone JM[1]
Pardes AM[1]
Morris TR[1]
Nuss CA[1]
Mauck RL[1]
Soslowsky LJ[1]
Kuntz AF[1]

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania Philadelphia, PA

Introduction

Rotator cuff tears affect millions of individuals each year, with an increased prevalence in the elderly population. Although advancements in surgical methods and rehabilitation protocols have improved clinical results, rotator cuff repair failure remains common.[1] To further improve surgical outcomes, rotator cuff repair augmentation has been studied, wherein scaffolds are used to aid in mechanical support at the time of surgery, and/or to deliver cells and/or biologic factors to the repair site. For example, local delivery of mesenchymal stem cells or tenocytes can increase collagen content and decrease inflammation at the repair site.[2-4] However, whether the success of such therapies is age-dependent is unknown. Therefore, the objective of this study was to determine the effects and mechanisms of action of autologous juvenile, adult, and aged tenocytes delivered using aligned nanofibrous scaffolds on healing tissue properties in our novel rat model of augmented rotator cuff repair.[5] Our hypotheses were: 1) Tenocyte-seeded scaffolds will increase collagen and cell organization at the repair site compared to scaffold only controls, resulting in enhanced tendon-bone healing with improved mechanical properties, and 2) tenocytes from juvenile rats will result in greater improvement of functional outcomes in cuff healing compared to adult or aged rats.

Methods

A total of 57 Fisher (F-344) rats were divided into three age groups: juvenile (4 weeks), adult (8 months) and aged animals (16 months). Animals underwent bilateral transection of their supraspinatus tendons and simultaneous harvest of the intra-articular biceps tendons. Biceps tendon cells were harvested by morselizing explant tissue and allowing cell migration onto tissue culture surfaces over 1 week. Cells were expanded in culture and split at confluence. At P2, cells from each donor were seeded onto an electrospun poly(δcaprolactone) (PCL) nanofibrous scaffold (3x5x0.5 mm) at $2x10^5$ cells/scaffold. Three weeks later, augmented supraspinatus repair was performed in which the right shoulder received a tenocyte-seeded scaffold while the left shoulder received an acellular scaffold as a control. Animals were sacrificed 8 weeks after the second surgery and frozen (for mechanical analysis, n = 12) or fixed in formalin (for histologic analysis, n = 6). One additional animal from each age group received Qtracker labeled cells bilaterally for cell tracking and was sacrificed at 1 week. Tissues were cryosectioned and processed for fluorescent imaging. *Tendon Mechanical Testing:* For testing, animals were thawed and the humerus was dissected with the supraspinatus intact. For local optical strain measurement, stain lines were placed on the tendon. Cross sectional area was measured using a custom laser device. Tensile testing was performed as: preload, preconditioning, stress relaxation, and ramp to failure. Stress was calculated as force divided by initial area and 2D Lagrangian strain was determined. *Histology:* Tendons were processed using paraffin procedures. Sagittal sections (7 um) were collected and stained with Hematoxylin–Eosin (H&E) or Safranin O-Fast Green (SafO). Cell density and cell shape were graded by three blinded investigators, using a scale of 1-3 (1 = low, 2 = moderate, 3 = high) for cellularity and 1-3 (1 = spindle shaped, 2 = mixed, 3 = rounded) for cell shape. SafO staining was quantified using ImageJ. Polarized light images were used to quantitate tendon organization as described.[6] Tissue mechanics, SafO quantification, and polarized light analysis were assessed using t-tests, comparing scaffold control and cell-seeded scaffold treatment within age groups. Histology scores were evaluated using a Mann-Whitney test. Significance was set at $p < 0.05$ (*) and trends at $p < 0.1$ (†); ** denotes $p < 0.01$.

Results

The presence of delivered tenocytes in the shoulder one week after surgery was confirmed via fluorescent imaging of Qtracker-labeled tenocytes. Concurrent in vitro culture of labeled cells demonstrated that tenocytes readily adhered to and colonized the PCL scaffolds (data not shown). Elastic and viscoelastic mechanical properties improved with cell-seeded scaffolds in both juvenile and aged animals when compared to scaffold-only controls. Specifically, in cell-seeded groups, stiffness and midsubstance modulus increased in juvenile animals, and midsubstance modulus increased in aged

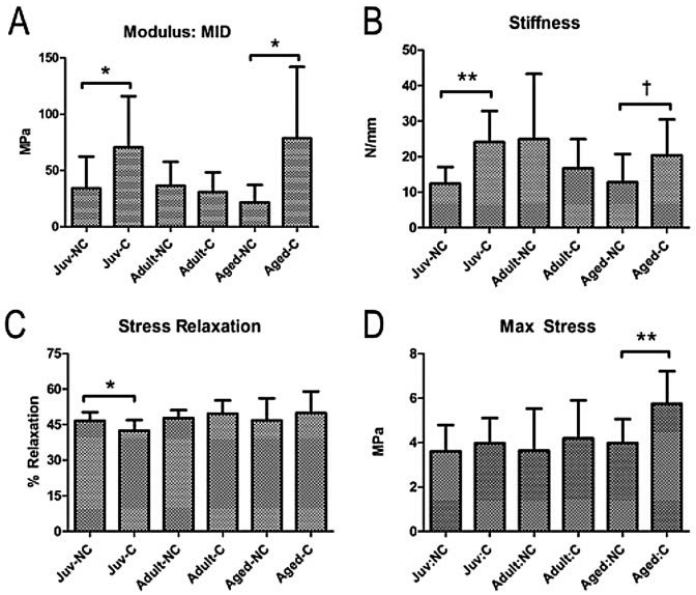

Figure 1. Augmenting repair with autologous cells caused (A) increased midsubstance modulus and (B) increased stiffness in both juvenile and aged animals, (C) decreased stress relaxation in juvenile animals, and (D) increased max stress in aged animals. (NC = Scaffold only, C = Cells).

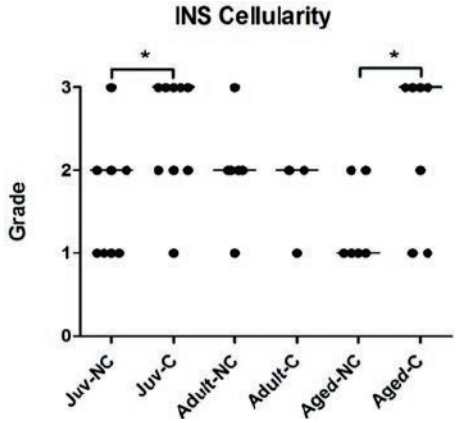

Figure 2. There was a significant increase in cellularity at the insertion of juvenile and aged tendons treated with cell-seeded scaffolds compared to scaffold-only controls.

animals, with a trend toward an increase in stiffness (Figure 1A-B). Similar improvements in insertion modulus were also noted (data not shown). Stress relaxation decreased in juvenile animals (Figure 1C), while maximum stress increased in aged animals (Figure 1D) relative to controls. Implantation of cell-seeded scaffolds also improved histological parameters. Specifically, cells were significantly more spindle-shaped in juvenile animals (insertion only, data not shown) and in aged animals (trend, midsubstance only, data not shown), while cellularity increased at the insertion in both juvenile and aged groups (Figure 2). Cell delivery decreased SafO staining in aged animals (data not shown), and increased collagen organization (decreased circular standard deviation) in both the insertion and midsubstance of aged animals, and in the insertion of juvenile animals (Figure 3). No changes were seen in any parameter in adult animals.

Discussion

Results demonstrate that delivery of autologous tenocytes to the healing supraspinatus is beneficial in juvenile and aged animals, with no effect in adult animals. As aged animals exhibit deteriorated tendon mechanical properties and healing potential,[7] a 44% increase in maximum stress with cell augmentation denotes a substantial improvement. Coupled with up to three-fold increases in tendon modulus and stiffness, the improvements in tendon mechanical strength for both aged and juvenile animals were striking. Increased numbers of cells at the insertion of treated animals demonstrates a more robust repair response, reflected in significant increases in collagen organization. These data support earlier matrix remodeling and increased collagen production after cell-augmented repair. While these findings support our first hypothesis, our second hypothesis was not substantiated. Surprisingly, both young and old animals benefitted similarly, yet no changes were seen in adult animals. Adult tendons are in relative "equilibrium" with regards to catabolic and anabolic processes, so additional tenocytes likely only sustain regular repair mechanisms. Conversely, juvenile tendons are

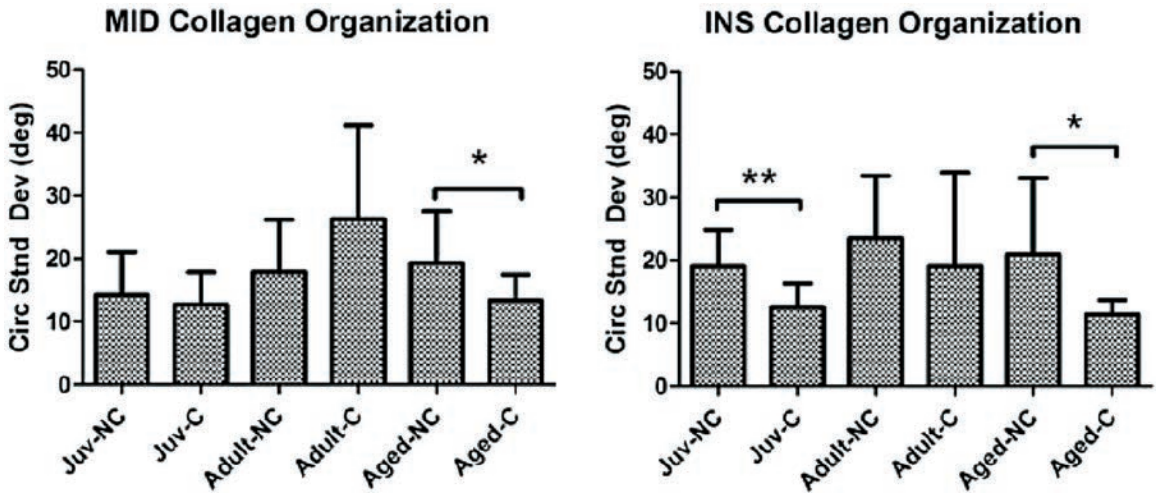

Figure 3. When comparing groups treated with cells to those without cells, collagen fiber organization was increased at the both the midsubstance and insertion in aged animals, and at the insertion in juvenile animals.

actively growing whereas aged tendons exhibit diminished cell activity and matrix turnover. In these "imbalanced" states, the addition of a supplemental cell population contributes substantially to the healing process. Further research will investigate precise mechanisms of action by which these cell populations improve tissue healing.

Significance

This research addresses regenerative medicine and musculoskeletal repair by using bioengineered scaffolds *in vivo* to improve tendon repair in a clinically relevant and well-established animal model. As this approach uses FDA approved materials and minimally manipulated autologous cell populations, it has the potential for rapid translation to clinical practice to address this important clinical problem. We have demonstrated the potential for autologous cell-seeded scaffolds to improve repairs in both the juvenile and aged population.

Acknowledgements

This study was supported by the UPenn Institute on Aging and the Penn Center for Musculoskeletal Disorders (P30 AR050950).

References

1. Galatz LM *et al.* *J Bone Joint Surg Am*, 2004.
2. Hernigou P *et al.* *Int Orthop*, 2014.
3. Chen JM *et al.* *Tissue Eng*, 2007.
4. Longo UG *et al.* *Br Med Bull*, 2010.
5. Beason DP *et al.* *J Shoulder Elbow Surg*, 2012.
6. Gimbel JA, *J Biomech*, 2004.
7. Plate JF *et al*. *Am J Sports Med*, 2014.