Published in final edited form as:

Wound Repair Regen. 2010 ; 18(5): 543–549. doi:10.1111/j.1524-475X.2010.00615.x.

Fetal Tendon Wound Size Modulates Wound Gene Expression and Subsequent Wound Phenotype

Benjamin J. Herdrich, MD^{1,3}, Enrico Danzer, MD^{1,3}, Marcus G. Davey, PhD³, Dustin M. Bermudez, MD^{1,3}, Antoneta Radu³, Liping Zhang³, Zhe Zhang, PhD³, Louis J. Soslowsky, PhD^{1,2}, and Kenneth W. Liechty, MD^{1,3,4}

¹ The University of Pennsylvania School of Medicine, Philadelphia, PA

² The McKay Orthopaedic Research Laboratory, Philadelphia, PA

³ The Children's Hospital of Philadelphia, Philadelphia, PA

⁴ The University of Mississippi Medical Center, Jackson, MS

Abstract

The fetal response to small tendon injury results in regenerative or scarless healing and is characterized by a markedly diminished cellular inflammatory response, lack of fibroplasia, and restoration of normal tissue architecture. We hypothesized that increasing fetal tendon wound size would lead to increased wound inflammation and a change from regenerative to reparative healing and scar formation. We created small or large tendon wounds in early gestation fetal sheep and used histology to assess tissue architecture, immunohistochemistry to assess the cellular inflammatory response, ovine specific gene microarrays, and real time RT-PCR to measure gene expression in response to injury. Small tendon wounds demonstrated a regenerative healing phenotype with orderly deposition of collagen fibers while large tendon wounds demonstrated disorderly collagen deposition consistent with scar formation. Small tendon wounds had few inflammatory cells at 7 and 28 days after injury, whereas the large wounds showed a significant inflammatory cell infiltrate at 7 days that resolved by 28 days. At 3 days, differential expression of genes involved in the response to injury and inflammation were seen between large and small tendon wounds. By real-time PCR at 7 days, large tendon wounds also had significantly increased expression of interleukin-6, interleukin-8, transforming growth factor- β 1, and transforming growth factor- β 3, compared to the small wounds. Increasing fetal tendon wound size results in increased proinflammatory gene expression inflammatory cell infiltration, and a change from regenerative to reparative healing. This model allows the process of regenerative healing to be examined without the confounding variable of gestational age.

Keywords

Regenerative Healing; Scar; Inflammation; Cytokine

INTRODUCTION

Following injury to the adult tendon there is a coordinated healing process that includes hemostasis, inflammation, proliferation, fibroplasia, and maturation which results in scar

Corresponding Author: Benjamin J. Herdrich, MD, 4 Maloney, 3400 Spruce St., Philadelphia, PA 19104, Phone: (215) 738-0420, Fax: (215) 662-7983, benjamin.herdrich@uphs.upenn.edu.

Reprints: Kenneth W. Liechty, MD, Department of Surgery, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, Phone: (601) 894-5050, Fax: (601) 815-3734, Liechty@email.chop.edu

formation (1–7). Tendon scar is biomechanically inferior to normal tendon, and complications following repair include tendon rupture and contracture formation, which highlight the result of scar formation in this tissue (1,8–12). Treatment strategies aimed at minimizing adhesions and contracture formation and to increase the strength of the healed tendon have the potential to prevent these complications and may dramatically impact the morbidity following tendon repair.

In contrast to the adult, fetal skin and tendon wounds have the capability to heal by regeneration with restoration of normal tissue architecture and do so with a lack of fibroplasia and a diminished inflammatory response (13–19). While these regenerative properties of fetal tissues have been known for decades, effective strategies to modify the adult response to injury have been lacking. Part of this limitation may be the reliance of previous studies comparing the fetal wound to the adult wound, where the developmental stage is different and may be a potential confounding variable in understanding the key contributions to the regenerative process.

We have shown that fetal skin wounds have low expression of the proinflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) following injury (20–21), and that absence of the anti-inflammatory cytokine interleukin-10 (IL-10) in the fetal skin promotes inflammation and scar formation following injury (22). In addition, the transforming growth factor- β (TGF- β) family of growth factors, which are important to many different cell functions, such as apoptosis, differentiation, epithelial-mesenchymal cell transformation, proliferation, and extracellular matrix (ECM) production (23), have been implicated in regulating the fetal response to injury (24). As development progresses from early gestation to the postnatal period, TGF- β 1, which is known to promote fibrosis, shows increasing expression in fetal dermis while TGF- β 3 shows decreasing expression (24). These studies suggest that fetal wounds have different levels of key growth factors and cytokines that may be important in promoting the scarless phenotype; however, the key contribution to scarless regeneration may be limited by the confounding variable of gestational age.

Increasing fetal skin wound size has been shown to result in a change from regenerative to reparative healing (25), however the mechanisms involved in this change are unknown. Small fetal tendon wounds have been shown to heal by regeneration with orderly deposition of collagen and restoration of normal tissue architecture (19). However, it is not known whether increasing fetal tendon wound size will result in a change in the healing phenotype from regeneration to scar formation. In this study, we hypothesized that increased fetal tendon wound size will result in increased production of proinflammatory cytokines, increased inflammatory cell infiltrate, and a change from regenerative to reparative healing. In addition, this would provide a consistent fetal model system, without the potentially confounding variable of developmental stage of the animal, to study the factors involved in regenerative versus scarless fetal healing.

METHODS

Animals

The animal handling and experimentation was approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia. Time dated pregnant ewes at 69–77 days gestation were used for the studies. Animals were sedated with ketamine 11 mg/kg IM, intubated, and anesthetized with inhaled isoflurane. Cefazolin 1 g IV was given prior to incision and oxytetracycline 0.06 mg/kg IM prior to extubation for antibiotic prophylaxis. A midline laparotomy and hysterotomy was performed to expose the fetus. A total of 19 fetuses were used in the study. The fetal extremity was exposed and transverse incisions made over the extensor surface of the forelimbs to expose the lateral extensor tendon. For

the small wounds, a transverse tenotomy incision was made with a scalpel encompassing 50% of the width of the tendon (Figure 1). For the large wounds, an excisional wound encompassing 50% of the width of the tendon and extending for 2mm in length was made (Figure 1). The wounds were marked with India ink, and the fetal skin incision was closed with absorbable interrupted sutures. The amniotic fluid was replaced with sterile normal saline containing 2 million units of penicillin-G for antimicrobial prophylaxis, and the uterus and abdominal incisions in the ewe were closed with absorbable suture. Analgesia was provided with buprenorphine 0.005mg/kg IM prior to extubation and flunixin meglumine 2.5mg/kg IM 4 hours post-operatively. The sheep were sacrificed at 3, 7 or 28 days, and the fetal tendons were harvested and processed for histology, immunohistochemistry, or RNA isolation.

Histology

A total of 8 fetuses were used in the histology and immunohistochemistry experiments. The fetal extensor tendons were harvested en block and fixed in 10% neutral buffer formalin (Sigma-Aldrich, St. Louis, MO) for greater than 72 hours prior to processing with a Leica 1050 histoprocessor (Leica Microsystems, Bannockburn, IL). 4µm paraffin serial sections were mounted on Fisher Plus slides (Fisher Scientific, Pittsburgh, PA) and incubated overnight at 52°F. Slides were deparaffinized in xylene for 10 minutes × 3 followed by graded ethanol rehydration (2 × 100%, 95%, 75%) to distilled water. Masson's Trichrome staining was performed as previously described (26).

Immunohistochemistry

From distilled water, the slides were immersed in 0.3% H₂O₂ in distilled water for 30 minutes at room temperature to quench endogenous peroxide. They were then rinsed well in distilled water. Antigen unmasking was done using target retrieval (pH 6.0, Dako, Carpinteria, CA) in the microwave (Ted Pella, Redding, CA) for 4 minutes and then cooled to room temperature. Slides were rinsed well in distilled water and serum specific blocking was done with 10% serum for 30 minutes at room temperature. The serum was tipped off and sections were incubated with primary antibody directed at CD45 (AbD Serotec, Oxford, UK, mouse anti-ovine at 1:100 dilution) overnight at 4°C. The following day, the primary antibody was tipped off and the slides washed in 0.1M PBS + Triton. Biotinylated specific secondary antibody (Vector Lab, Burlingame, CA, horse anti-mouse at 1:200 dilution) was applied for 30 minutes at room temperature. The secondary antibody was tipped off, and the slides were rinsed in PBS + Triton followed by ABC Complex (Avidin-Biotin, Vector Lab, Burlingame, CA) applied for 30 minutes at room temperature. Slides were washed in PBS + Triton, and the developer with chromogenic substrate (ImmPACT DAB, Vector Lab, Burlingame, CA) was applied for 1–2 minutes. Slides were then rinsed in distilled water and counterstained with Harris Hematoxylin (Fisher Scientific, Pittsburgh, PA). Sections were then dehydrated through graded alcohol series for 2 minutes each (2 × 70%, 95%, 100%), clarified in xylene, and a cover slip applied in Permount (Fisher Scientific, Pittsburgh, PA).

RNA isolation and Ovine Specific Gene Microarray

A total of 4 fetuses were used in the gene microarray experiments. A 5 mm section of the lateral extensor tendon centered on the tendon wound was harvested 3 days after injury and flash frozen in liquid nitrogen (small n=4, large n=3). Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and then labeled with the incorporation of fluorescent dCTP. The mixed probes were then hybridized to the oligonucleotide ovine micorarrays (Agilent Technologies, Foster City, CA). The background-subtracted signal intensities were imported into R statistical environment (www.r-project.org) for statistical

analysis, after which lowess-normalization of all 15,008 probes was performed across samples.

Since more than 80% of the probes correspond to sheep genes that are not functionally annotated, we mapped them to human genes through UniGene database (www.ncbi.nlm.nih.gov/unigene), which clusters genes across species based on sequence similarity. As a result, 9,103 probes were mapped to 7,384 unique human genes. The full version of the probe annotation file is available as supplementary material. Using human genes as medium, 31 probes were related to “response to wounding” (GO:0009611) and 175 were related to “inflammatory response” (GO:0006954) according to Gene Ontology (www.geneontology.org).

Real Time RT-PCR

A total of 7 fetuses were used in the real-time RT-PCR experiments. Each fetus received a small tendon wound on one forelimb and a large tendon wound on the contralateral forelimb. A 5 mm section of the lateral extensor tendon centered on the tendon wound was harvested 7 days after injury and flash frozen in liquid nitrogen. The harvest method minimized the amount of normal tendon tissue surrounding the wound that was included in the sample. Total RNA was isolated using TRIzol Reagent and single-strand cDNAs synthesized from total RNA as described above. Comparative C_T Real-Time PCR amplification was performed in triplicate on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using the TaqMan Fast Universal PCR Master Mix and Custom TaqMan® Gene Expression Assays. Oligonucleotide primers and probes for RT-PCR were designed using File Builder Software (Applied Biosystems, Foster City, CA) according to mRNA sequences obtained from Genbank™. The following primers and probes were used: ovine IL-6 (primers) forward 5'-AGGAAAAAGATGGATGCTTCCA-3', reverse 5'-GACCAGCAGTGGTTTTGATCAA-3', (probe) 5'-CTGGGTTCAATCAGGCGA-3', (reference number) NM_001009392; ovine IL-8 (primers) forward 5'-TGCTCTCTTGGCCGCTTT-3', reverse 5'-TTGACAGAACTGCAGCTTCACA-3', (probe) 5'-CTGCTCTCTGCAGCTC-3', (reference number) NM_001009401; ovine TGF-β1 (primers) forward 5'-AAGCGGAAGGGCATCGA-3', reverse 5'-CGAGCCGAAGTTTGGACAAA-3', (probe) 5'-CCATCCGCGGTCAGA-3', (reference number) NM_001009400; and ovine TGF-β3 (primers) forward 5'-GCCGGGTGAGCACATAGC-3', reverse 5'-CGCGTGGGCAGGTTCTT-3', (probe) AGCAGCGGTATATCG, (reference number) AY656798. Eukaryotic 18S rRNA (Part Number 4319413E, Applied Biosystems, Foster City, CA) is used as endogenous control. Cycling conditions were as followed: 95°C for 20s followed by 40 cycles of 95°C for 3s, 60°C for 30s.

Statistics

Individual genes were normalized to 18s and RQ calculated relative to values obtained from an age matched uninjured tendon. All samples were conducted in triplicate. Biologic replicates were averaged, and RQ values are presented as mean±SEM. The data was not normally distributed; therefore, RQ values were analyzed with a Wilcoxon signed-rank test using p<0.05 to denote significance.

RESULTS

At 7 days after injury, Trichrome staining of the small tendon wounds (n=3) demonstrated normal tissue architecture with orderly collagen fiber orientation (Figure 2a), consistent with regenerative or scarless healing. In contrast, the large tendon wounds (n=7) all demonstrated disorderly deposition of collagen consistent with scar formation (Figure 2b). At 28 days

after injury, the small tendon wounds (n=3) demonstrated normal tissue architecture (Figure 2c), whereas the large tendon wounds (n=3) demonstrated persistent abnormal collagen fiber orientation and tissue architecture as well as remodeling of the scar.

At 7 days after injury (n=3), immunohistochemistry for CD45 demonstrated very few CD45+ inflammatory cells in the small wounds (Figure 3a). In contrast, the large tendon wounds (n=7), demonstrated a significant inflammatory infiltrate, with many CD45+ cells seen in all samples at 7 days (Figure 3b). By 28 days, there were still very few CD45+ inflammatory cells in the small tendon wounds (Figure 3c), and the inflammatory cell infiltrate had resolved in the large wounds (figure 3d).

Principal components analysis (PCA) using all probes indicated that the global gene expression patterns were distinguishable between large and small wounds (Figure 4). PCA identified three principal components that accounted for more than 80% of the total variance of the data set. These components were plotted for individual tendon wounds and demonstrate that small wounds (regenerative wounds) have a similar gene expression profile that is different from large wounds (scar forming wounds). PCA has been used in analyzing microarray data as it has the ability to reduce the dimensionality and identify patterns of large data sets. For instance, it has been shown to be useful in characterizing lung cancer subtypes and changes in gene expression profiles in mesothelial cell lines exposed to pathogenic materials (27–28).

From the 15,008 sheep array probes, we identified 218 over-expressed and 56 under-expressed probes in the large wounds compared to the small wounds (more than two-fold change and student's t-test $p < 0.05$). The estimated number of false positives in these probes was 19 according to a permutation procedure. Analysis using DAVID Functional Annotation (david.abcc.ncifcrf.gov) suggested the enrichment of biologically relevant genes in these probes. In addition, the two gene groups of interest, “response to wounds” and “inflammatory response”, from the Gene Ontology database, demonstrated that both of these groups of genes were significantly over-expressed in large wounds in general although the differential expression varied among individual genes (Figure 5).

Real-time RT-PCR for specific genes within these gene groups demonstrated significantly increased gene expression of multiple cytokines involved in the inflammatory and wound response 7 days after wounding in the large wounds compared to the small wounds. In large (n=7) versus small (n=7) tendon wounds there was a 79-fold increase in IL-6 gene expression ($RQ = 136.9 \pm 83.2$ vs. 1.7 ± 0.5 , $p < 0.05$, Figure 6a), a 652-fold increase in IL-8 gene expression ($RQ = 1585.3 \pm 851.0$ vs. 2.4 ± 1.5 , $p < 0.05$, Figure 6b), a 65-fold increase in TGF- β 1 gene expression ($RQ = 1290.2 \pm 569.2$ vs. 20.0 ± 8.4 , $p < 0.05$, Figure 6c), and a 58-fold increase in TGF- β 3 gene expression ($RQ = 493.8 \pm 245.8$ vs. 8.5 ± 4.2 , $p < 0.05$, Figure 6d).

DISCUSSION

In this study, we have shown that increasing fetal tendon wound size results in increased production of proinflammatory and profibrotic growth factors and cytokines, increased inflammatory cell infiltrate, and a change from regenerative to reparative healing. This shows that a robust inflammatory response can occur in the fetus, is associated with increasing wound size, and suggests a central role of inflammation in the mechanism of scar formation. Our study does not prove that inflammation is causally linked to the transformation from regenerative healing to scar formation; however, we hypothesize that this is the case.

Inflammation is a complex biologic process with multiple participating cell types, growth factors, and cytokines. The production of proinflammatory factors and recruitment of

monocytes, macrophages, and PMNs to the wound results in increased production and release of proinflammatory growth factors and cytokines by these recruited cells, which serves to amplify the inflammatory response. In addition, these recruited inflammatory cells produce reactive oxygen species (ROS) and increased oxidative stress in the wound. This may also lead to further tissue injury, resulting in increased production of proinflammatory cytokines and growth factors, and amplification of the inflammatory response. The role of ROS and the composition of the inflammatory cell infiltrate in mediating the transition from regenerative to reparative healing are not known. However, we believe that with increasing wound size, an exuberant inflammatory response likely promotes scar formation through a number of downstream mechanisms, such as regulating gene expression within the wound, affecting migration of different cell types to the wound, and affecting the function of cells present within the wound. Further studies are needed to prove whether inflammation is causally linked to the conversion from regenerative healing to scar formation and define what are the important biologic mechanisms affecting this conversion.

The complexity of the inflammatory response must be considered when attempting to modulate inflammation in a wound with the goal of promoting regenerative healing and minimizing scar formation. In order to develop effective strategies of promoting regenerative healing in the adult while avoiding deleterious effects on healing that can be seen with immunosuppression, a more detailed understanding is needed of how specific pathways within the inflammatory response affect the process of scar formation. One of the limitations in doing such research has been that previous studies have compared fibrotic healing with regenerative healing at different stages of development (adult versus fetal). This introduces a major confounding variable; therefore, when differences are found, it is very difficult to identify what is intrinsic to the process of regenerative healing or scar formation and what is simply a consequence of developmental stage. Our model of small and large tendon wounds in the fetal sheep, offers a large animal model of fetal regenerative healing and fetal scar formation where developmental stage is controlled; therefore, the biologic mechanisms controlling these processes can be investigated.

The finding of increased gene expression of both TGF- β 1 and β 3 isoforms illustrates the importance of controlling for developmental stage. TGF- β 1 and TGF- β 3 have been studied with regards to their roles in wound healing and scar formation. TGF- β 3 has been regarded as anti-fibrotic, as it was elevated in comparison to TGF- β 1 in fetal wounds, and not elevated in adult wounds relative to TGF- β 1. TGF- β 3 has been shown to reduce scarring in a rat cutaneous wound model, and a number of studies support TGF- β 1 as a profibrotic cytokine that is decreased in fetal wounds thus suggesting that an absence of TGF- β 1 plays a role in fetal regenerative healing (25,29–31). TGF- β 1 and TGF- β 3 have both been shown to have gene expression levels that vary with gestational age, and several studies have demonstrated different cellular responses of fetal versus adult fibroblasts to TGF- β (24,32–33). Therefore, any studies of TGF- β and wound healing must be considered in the context of the stage of development. Our study demonstrates that both TGF- β 1 and TGF- β 3 are significantly upregulated in the large tendon wounds at day 7, which will ultimately heal by scar formation. Small fetal tendon wounds at the same gestational age do not demonstrate this same amplification of gene expression following wounding. Upregulation of TGF- β 1 in our fetal model of scar formation is consistent with it being physiologically important in the process of fibroplasia. However, TGF- β 3 was also similarly upregulated in the large tendon wounds, which is in disagreement with studies that suggest that TGF- β 3 can act to reduce scar, and the ratio of the different TGF- β isoforms is what drives fibroplasia (27,29). Further studies are needed to better define the actions of TGF- β 1 and TGF- β 3 and link those actions to wound healing phenotype. Future investigation should use a model that controls for gestational age as in this study.

This study clearly demonstrates that there is regenerative healing in the small tendon wounds and scar formation in the large wounds implicating wound size as a mediator of scar formation. One caution in interpreting this study is that it does not address whether regenerative healing leads to a better functional outcome. It is reasonable to hypothesize that regenerative healing would lead to improved biomechanical properties of the healed tendon compared to scar formation. Previous studies have suggested that regenerative fetal healing in tendon and dermis still results in inferior biomechanical properties when compared to unwounded tissue; however, these studies were limited by only testing early time points (25,34). Additional studies that include long time points that allow for complete healing and remodeling of the wound are needed to fully address this question.

By studying the biologic processes controlling regenerative healing and scar formation in one consistent model system, it may be possible to identify novel therapeutic targets to promote regenerative healing in the adult and minimize the morbidity of pathologic fibrosis.

The transcriptomic data from microarray experiments revealed that the global gene expression patterns were different for our large and small tendon wounds (Figure 4). This demonstrates that wound size and phenotype (regenerative healing vs. reparative healing) are associated with a reproducible gene expression profile. Not only can this method be used as a marker of regenerative healing, but it also has the potential to identify key genes that could be targeted therapeutically to promote regenerative healing and minimize scar formation. While further investigation into the importance and biology of specific genes is needed, many genes differentially expressed between large and small wounds do have known biological significance. For example, the expression of ACAN, a gene encoding an integral part of cartilage, was 178% higher in small wounds than in large wounds. Validation and follow-up analysis of the focus genes will be included in future investigation.

In conclusion, our study has demonstrated that expression of proinflammatory cytokines and recruitment of inflammatory cells is associated with conversion from regenerative healing to scar formation. We have demonstrated upregulation of both the cellular inflammatory response and proinflammatory gene expression with increasing wound size. Furthermore, microarray data has allowed us to investigate the expression of a large number of genes across the entire genome. Future investigation in this model has the potential to identify therapeutic targets to promote regenerative healing in the adult.

Acknowledgments

This research was funded in part by grant support from the NIH/NIAMS supported Penn Center for Musculoskeletal Disorders (AR050950).

ABBREVIATIONS

ECM	Extracellular Matrix
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
PCA	Principal Components Analysis
ROS	Reactive Oxygen Species
TGF-β	Transforming Growth Factor- β

References

1. Lin TW, Cardenas L, Soslowsky LJ. Biomechanics of tendon injury and repair. *J Biomech.* 2004; 37(6):865–77. [PubMed: 15111074]
2. Fenwick SA, Hazleman BL, Riley GP. The vasculature and its role in the damaged and healing tendon. *Arthritis Res.* 2002; 4(4):252–60. [PubMed: 12106496]
3. Gelberman RH, Chu CR, Williams CS, Seiler JG 3rd, Amiel D. Angiogenesis in healing autogenous flexor-tendon grafts. *J Bone Joint Surg Am.* 1992; 74(8):1207–16. [PubMed: 1383229]
4. Gomez, M. The physiology and biochemistry of soft tissue healing. In: Griffin, LY., editor. *Rehabilitation of the Injured Knee.* St. Louis: Mosby; 1995. p. 34-44.
5. Grinnell F. Fibronectin and wound healing. *J Cell Biochem.* 1984; 26(2):107–16. [PubMed: 6084665]
6. Woo, SL.; An, K.; Frank, C.; Livesay, G.; Ma, A.; Zeminski, J.; Wayne, J.; Myers, B. Anatomy, biology, and biomechanics of tendon and ligament. In: Buckwalter, JA.; Einhorn, TA.; Simon, SR., editors. *Orthopaedic Basic Science: Biology and Biomechanics of the Musculoskeletal System.* Rosemont: American Academy of Orthopaedic Surgeons; 2000. p. 582-616.
7. Woo SL, Debski RE, Zeminski J, Abramowitch SD, Saw SS, Fenwick JA. Injury and repair of ligaments and tendons. *Annu Rev Biomed Eng.* 2000; 2:83–118. [PubMed: 11701508]
8. Frank C, McDonald D, Bray D, Bray R, Rangayyan R, Chimich D, Shrive N. Collagen fibril diameters in the healing adult rabbit medial collateral ligament. *Connect Tissue Res.* 1992; 27(4): 251–63. [PubMed: 1576825]
9. Frank C, McDonald D, Shrive N. Collagen fibril diameters in the rabbit medial collateral ligament scar: a longer term assessment. *Connect Tissue Res.* 1997; 36(3):261–9. [PubMed: 9512894]
10. Frank C, Woo SL, Amiel D, Harwood F, Gomez M, Akeson W. Medial collateral ligament healing. A multidisciplinary assessment in rabbits. *Am J Sports Med.* 1983; 11(6):379–89. [PubMed: 6650715]
11. Manske PR. History of flexor tendon repair. *Hand Clin.* 2005; 21(2):123–7. [PubMed: 15882591]
12. Tang JB. Clinical outcomes associated with flexor tendon repair. *Hand Clin.* 2005; 21(2):199–210. [PubMed: 15882599]
13. Longaker MT, Whitby DJ, Adzick NS, Crombleholme TM, Langer JC, Duncan BW, Bradley SM, Stern R, Ferguson MWJ, Harrison MR. Studies in fetal wound healing: VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. *J Pediatr Surg.* 1990; 25:63–69. [PubMed: 2299547]
14. Rowlatt U. Intrauterine wound healing in a 20-week human fetus. *Virchows Arch A Pathol Anat Histol.* 1979; 381(3):353–61. [PubMed: 155931]
15. Burrington JD. Wound healing in the fetal lamb. *J Pediatr Surg.* 1971; 6:523–8. [PubMed: 5166565]
16. Adzick NS, Harrison MR, Glick PL, Beckstead JH, Villa RL, Scheuenstuhl H, Goodson WH III. Comparison of fetal, newborn and adult wound healing by histologic enzyme-histochemical and hydroxyproline determination. *J Pediatr Surg.* 1985; 20:315–9. [PubMed: 4045654]
17. Adzick, NS.; Longaker, MT. *Fetal Wound Healing.* New York: Elsevier Scientific Press; 1992.
18. Krummel TM, Nelson JM, Diegelmann RF, Lindblad WJ, Salzberg AM, Greenfield LJ, Cohen IK. Fetal response to injury in the rabbit. *J Pediatr Surg.* 1987; 22:640–4. [PubMed: 3302202]
19. Beredjikian PK, Favata M, Cartmell JS, Flanagan CL, Crombleholme TM, Soslowsky LJ. Regenerative versus reparative healing in tendon: a study of biomechanical and histological properties in fetal sheep. *Ann Biomed Eng.* 2003; 31(10):1143–52. [PubMed: 14649488]
20. Liechty KL, Crombleholme TM, Adzick NS. Decreased IL-6 in scarless human fetal repair. *Cytokine.* 2000; 12:671–6. [PubMed: 10843743]
21. Liechty KW, Crombleholme TM, Cass DL, Martin B, MS, Adzick NS. Diminished Interleukin-8 (IL-8) production in the fetal wound healing response. *J Surg Res.* 1998; 77:80–4. [PubMed: 9698538]
22. Liechty KW, Kim HB, Adzick NS, Crombleholme TM. Fetal wound repair results in scar formation in interleukin-10 (IL-10) deficient mice in a syngeneic murine model of scarless fetal wound repair. *J Pediatr Surg.* 2000; 35:866–73. [PubMed: 10873028]

23. Schiller M, Javelaud D, Mauviel A. TGF- β -induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. *J Dermatol Sci*. 2004; 35:83–92. [PubMed: 15265520]
24. Chen W, Fu X, Shili Ge, Sun T, Zhou G, Jiang D, Sheng Z. Ontogeny of expression of transforming growth factor- β and its receptors and their possible relationship with scarless healing in human fetal skin. *Wound Rep Reg*. 2005; 13:68–75.
25. Cass DL, Bullard KM, Sylvester KG, Yang EY, Longaker MT, Adzick NS. Wound size and gestational age modulate scar formation in fetal wound repair. *J Pediatr Surg*. 1997; 32:411–5. [PubMed: 9094005]
26. McElroy, DA. Connective tissue. In: Prophet, EB.; Mills, B.; Arrington, JB.; Sobin, LH., editors. *Laboratory Methods in Histotechnology*. Washington, D.C: Armed Forces Institute of Pathology; 1994. p. 132-3.
27. Watanabe T, Miura T, Degawa Y, Fujita Y, Inoue M, Kawaguchi M, Furihata C. Comparison of lung cancer cell lines representing four histopathological subtypes with gene expression profiling using quantitative real-time PCR. *Cancer Cell Int*. 2010; 10:2. [PubMed: 20142997]
28. Hillegass JM, Shukla A, MacPherson MB, Bond JP, Steele C, Mossman BT. Utilization of gene profiling and proteomics to determine mineral pathogenicity in a human mesothelial cell line (LP9/TERT-1). *J Toxicol Environ Health A*. 2010; 73:423–36. [PubMed: 20155583]
29. Shah M, Foreman DM, Ferguson MW. Neutralization of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci*. 1995; 108:985–1002. [PubMed: 7542672]
30. Lin RY, Sullivan KM, Argenta PA, Meuli M, Lorenz HP, Adzick NS. Exogenous transforming growth factor-beta amplifies its own expression and induces scar formation in a model of human fetal skin repair. *Ann Surg*. 1995; 222(2):146–54. [PubMed: 7639582]
31. Soo C, Beanes SR, Hu FY, Zhang X, Dang C, Chang G, Wang Y, Nishimura I, Freymiller E, Longaker MT, Lorenz HP, Ting K. Ontogenetic transition in fetal wound transforming growth factor- β regulation correlates with collagen organization. *Am J Pathol*. 2003; 163(6):2459–76. [PubMed: 14633618]
32. Pratsinis H, Giannouli CC, Zervolea I, Psarras S, Stathakos D, Kletsas D. Differential proliferative response of fetal and adult human skin fibroblasts to transforming growth factor- β . *Wound Rep Regen*. 2004; 12(3):374–83.
33. Rolfe K, Richardson J, Vigor C, Irvine LM, Grobbelaar AO, Linge C. A role for TGF- β 1-induced cellular responses during wound healing of the non-scarring early human fetus. *J Invest Dermatol*. 2007; 127(11):2656–67. [PubMed: 17687390]
34. Julia MV, Albert A, Morales L, Miro D, Sancho MA, Garcia X. Wound healing in the fetal period: the resistance of the scar to rupture. *J Pediatr Surg*. 1993; 28(11):1458–62. [PubMed: 8301458]

Figure 1.

Small and large tendon injury models. The small incisional wound was made perpendicular to the tendon extending 50% of the width of the tendon. The large excisional wound resulted in a defect extending 50% of the width of the tendon and 2mm in length.

Figure 2.

Masson's Trichrome staining of small and large tendon wounds. 7 days after injury (A) the small tendon wounds demonstrated normal tissue architecture while (B) the large tendon wounds demonstrated disordered deposition of collagen fibers consistent with scar formation. At 28 days after injury (C) the small wounds continued to show normal tissue architecture. (D) The large wounds demonstrated disorganized collagen fibers with some remodeling and maturation of the scar. India ink marks the wound site. Scale bars equal 100µm.

Figure 3.

CD45 immunohistochemistry of small and large tendon wounds. 7 days after injury (A) there was a paucity of CD45+ inflammatory cells in the small tendon wounds while (B) there was infiltration of CD45+ inflammatory cells to the site of the wounds. At 28 days after injury, (C) the small tendon wounds continued to show a paucity of inflammatory cells, and (D) the inflammatory cell infiltrate had resolved in the large wounds. Scale bars equal 100 μ m.

Figure 4.

Principal components analysis (PCA) on three pairs of large and small wounds from the same fetuses demonstrates that there were global gene expression patterns specific to large and small wounds. PCA was performed on the microarray data set generated for large and small fetal tendon wounds with the `prcomp()` function in R to decompose the total variance, and the top three principal components plotted in this figure altogether accounted for more than 80% of the total variance. The large markers correspond to large wounds, and the small markers correspond to small wounds. Different colors are used to identify different fetuses. Grouping of data based on wound size indicates that there are similar patterns of gene expression in large wounds that differ from those seen in small wounds.

Figure 5.

These box plots indicate that the genes related to “response to wounding” and “inflammatory response” Gene Ontology terms have higher expression level in large wounds in general. The y-axis of this figure represents the log2 ratio of average expression in large and small wounds. The boxes represent the distribution of the log2 ratios in each group. Each probe is marked as a diamond whose locations were jittered horizontally to reduce overlapping. By average, the expression of “response to wounding” genes were 29.2% higher and the expression of “inflammatory response” genes were 28.9% higher in large wounds than in small wounds. Both groups of genes were more favorably expressed in the large wounds compared to the genes in neither groups ($p < 0.005$, student’s t test).

Figure 6. Real Time RT-PCR in small and large tendon wounds at 7 days. There was a significant increase in the gene expression in large tendon wounds compared to small wounds for (A) IL-6 ($p < 0.05$), (B) IL-8 ($p < 0.05$), (C) TGF- β 1 ($p < 0.05$), and (D) TGF- β 3 ($p < 0.05$).