Dehydrated Placental Membrane Improves Repair of Achilles Tendon Injury in a Diabetic Animal Model

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Statement of Purpose

Achilles tendon ruptures occur in approximately 18 per 100,000 people[1], with the incidence of acute Achilles tendon ruptures increasing[2–4]. The healing of tendon injuries is often plagued by significant scar formation and compromised biomechanical function[5-7]. For diabetics, these injuries are further complicated by alterations to the extracellular matrix of the tendon, poor circulation, and delayed wound healing[8,9]; consequently, complications and re-rupture rates for diabetic patients are reported higher than the typical patient population[10]. Placental derived membranes, specifically dehydrated human amnion/chorion membranes (Figure 1), have been utilized clinically as an adhesion barrier, and these membranes have been shown to reduce scarring and aid in tissue repair. Animal studies utilizing amnion derived cells and tissues to augment tendon repair have shown a reduction in healing time and overall improvements in tendon structure[11,12]. Initial clinical evidence has indicated that patients with tendon-pathologies treated with placental-derived grafts report reduced pain and improved function[13–15]. The purpose of this study was to evaluate the effect of dehydrated amnion/chorion membranes (dACM) on tendon repair in a diabetic model with impaired healing.

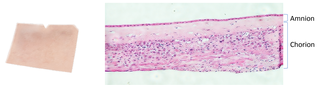

Figure 1: Dehydrated human amnion chorion membrane (dACM) *(left)*. H&E image of dehydrated amnion/chorion membranes *(Right)*.

Methodology & Hypothesis

We hypothesized that the use of dACM as a tendon wrap would result in the up-regulation of factors important for tendon repair and healing and the repaired tendon would have improved mechanical properties. To test this hypothesis we utilized a Type-2 insulin dependent animal model, the BBZDR/WOR rat. The protocol for this study was reviewed and approved by the UMASS Medical School IACUC (protocol # A-2537-15). Prior to the generation of the tendon injury, fasting blood glucose levels were taken; confirmation of diabetes was based on blood glucose levels of over 140mg/dL following overnight fasting.
BBZDR/WOR animals were randomly divided into one of two groups: tendon repair with dACM wrap or control (repair with no wrap of tendon). For each group, animals were sacrificed at either 14 or 28 days and the contralateral tendon served as a sham control (n=15 per group, n=30 total). At 14 days, 5 animals per group were sacrificed for cell migration analysis and immunohistochemical staining (n=10), and at 28 days 10 animals per group were sacrificed per group (n=20) with 5 used for cell migration analysis and immunohistochemical staining and 5 used for mechanical testing. For both mechanical testing and migration analysis, Statistical analysis was conducted using a one-way ANOVA with a post-hoc Tukey test where p<0.05 was considered significant

Procedure

Animals were anesthetized via inhalation of isoflurane, and both hind limbs where shaved and swabbed with betadine and 70% ethanol three times. A 1-cm longitudinal midline posterior incision was made to expose the Achilles tendon, and the fascia surrounding the Achilles tendon was exposed. Upon exposure, surrounding fascia was dissected away (all groups), and the sham group was immediately closed. For the control and dACM treated groups, a full thickness tendon injury was generated with a scalpel through the central region of the Achilles tendon. The Achilles tendon was then immediately repaired using the modified Kessler method with 6/0 polypropylene sutures. dACM treated tendons were then wrapped with a 0.5 cm x 0.5 cm section of dACM around the injured portion of the tendon with the stromal side of the graft oriented in contact with the tendon (control tendons were left unwrapped). The incision was then closed with 5/0 nylon sutures (Ethicon, USA) and postoperatively animals were returned to normal cage activity.

Results

By 28 days, noticeable differences were observed in the overall integrity of the repaired tendons in each group. Interestingly, 20% (3/15) of the control tendons re-ruptured compared with 0% of the dACM treated tendons. Biomechanical tensile testing of tendons (Figure 2) showed increases in the maximum tensile load for dACM treated tendons compared to controls, and a significant increase in stiffness compared to controls 31.67±19.43 N/mm verses 12.83±6.87 N/mm, respectively (p<0.05).
Retrieved tendons were evaluated for morphological changes based on H&E stained sections at both 14 and 28 days (Figure 3). Qualitatively, dACM treated tendons resulted in increased cell density and improvements in tendon fiber organization. However, when compared to uninjured tendons (sham) at both 14 and 28 days, both dACM and control groups were structurally inferior in terms of fiber structure and arrangement. Evaluation of DAPI stained sections from retrieved tendons at 14 and 28 days found dACM treated tendons resulted in significant increases in cell migration compared to control and sham groups (Figure 4). Immunofluorescent analysis of retrieved tendons (Figure 5) at 14 days showed that compared to the sham group, unwrapped tendons (control) had increased levels of COX2, IL-1β, IL-6, MMP-2, TGF-β1, and TIMP1. When comparing dACM treated tendons to control tendons at 14 days, IL-6 and MMP-2 levels were reduced in the dACM groups while TGF-β1 and TIMP1 were elevated in dACM treated tendons. At 28 days, compared to the sham group unwrapped tendons (control) still had slightly increased levels of COX2, IL-1β, TGF-β1, and TIMP1. When comparing dACM treated tendons to unwrapped tendons (control) at 28 days, COX2, IL-1β, TGF-β1, and TIMP1 levels were lower in dACM treated groups (Figure 6). Interestingly, Scleraxis, which is known to be critical for certain aspects of tenogenesis and repair, was consistently increased in dACM groups at both 14 and 28 days compared to both control and sham groups (Figure 7).

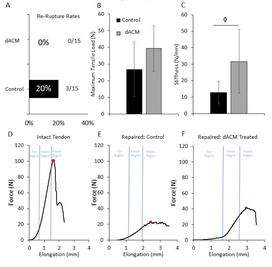

Figure 2: Biomechanical Properties of repaired Achilles tendons at 28 days post injury. (A) By 28 days control tendons had a 20% re-rupture rate, while no ruptures were observed with dACM treated tendons. (B) Maximum tensile load for both control and dACM treated tendons retrieved 28 days post injury. (C) Stiffness of control and dACM treated tendons 28 days post injury. For B-C averages are presented and error bars represent standard deviation, N=5. Representative force-displacment curves for non-injured sham tendons (intact) (D), repaired control tendons (E), and repaired dACM treated tendons at 28 days post-injury. For D-F the red dot indicates the maximum failure load. ◊ represents significance (p<0.05) compared to control groups

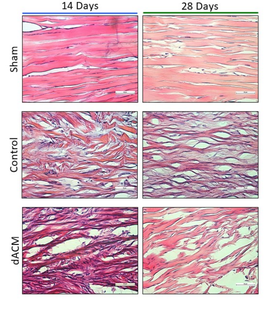

Figure 3: Representative H&E stained images of retrieved tendons at 14 (left column) and 28 days (right column) post-injury. Images taken with a 40x objective, scale bar represents 50µm.

Results

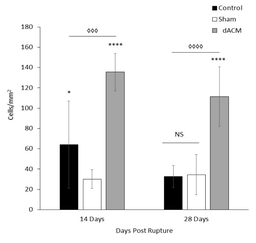

Figure 4: Cell migration to the repair site at 14 and 28 days post injury. Cell density was measured using DAPI stained sections. At 14 days, dACM treated tendons had a significantly higher cell density compared to both sham and control tendons. By 28 days, there was no observable difference in the cell density between control and sham groups, while the dACM treated group had a significantly higher cell density compared to both control and sham groups. * represents significance compared to shams, while ◊ represents significance compared to control groups. * p<0.05, **** p< 0.0001, ◊◊◊ p< 0.001, and ◊◊◊◊ p<0.0001

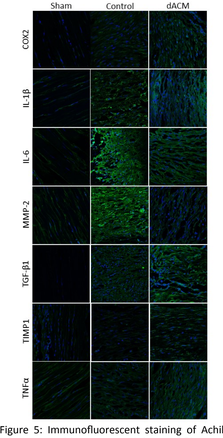

Figure 5: Immunofluorescent staining of Achilles tendons at 14 days post-injury. Biomarkers (green) expressed at 14 days post injury were assessed using IMF for control/unwrapped tendons (center column), dACM treated tendons (right column), and sham groups (left column). Images were taken using a 63x oil immersion objective.

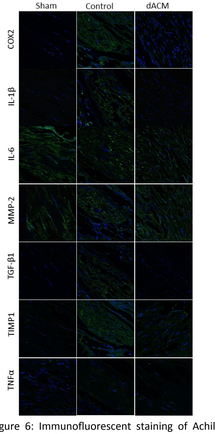

Figure 6: Immunofluorescent staining of Achilles tendons at 28 days post-injury. Biomarkers (green) expressed at 28 days post injury were assessed using IMF for control/unwrapped tendons (center column), dACM treated tendons (right column), and sham groups (left column). Images were taken using a 63x oil immersion objective.

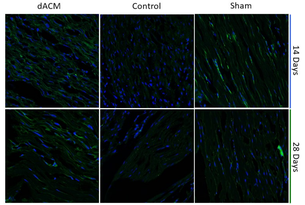

Figure 7: Immunofluorescent staining of Scleraxis (Scx), a transcription factor involved in tendon repair at 14 and 28 days. dACM treated tendons resulted in upregulation of Scx in tendons at days 14 and 28 as judged by immunofluorescence. In contrast, there is limited staining for Scx in the unwrapped and sham controls. Images were taken using a 63x oil immersion objective.

Analysis & Discussion

DM increases the risk of tendon injury and inhibits the ability of tendons to heal normally[8-10]. The results of this study confirm that injured diabetic tendons showed a significant delay in healing as determined by reduced biomechanical properties at 28-days post injury, reduced cell migration, and an inadequate proliferative healing phase as determined by the delay in the expression of biomarkers such as COX2, IL-1β, IL-6, TGF-β1, and TIMP1.
Human amniotic allografts have been shown to improve tendon repair in both preclinical and early clinical reports[11-15]. The results of this study are consistent with previous findings where tendons treated with amniotic tissues showed improvements in biomechanical properties[16-17]. This study is the first to evaluate the effect of an amniotic allograft in a delayed diabetic model of tendon healing, and the first study to evaluate dACM grafts as an adjunct for Achilles tendon repair. Placental derived grafts have been reported to contain numerous growth factors and cytokines thought to support tendon healing including PDGF-BB, IGF-1, TGF-β1, and bFGF[16,17]. Placental tissues have also been shown to improve cell proliferation and migration in a variety of cell types and promote angiogenesis[17].
The changes in biomarker expression between dACM treated and unwrapped tendons observed in this study suggest one mechanism for increased mechanical integrity with dACM-wrapped tendons may be related to the progression into the repair phase of tendon healing. Of particular interest is Scleraxis (Scx), which was increased in dACM treated tendons at both 14 and 28 days compared to controls. Scx is a basic helix-loop-helix transcription factor which is critical for certain aspects of tenogenesis and repair, and is expressed in both tendon and ligament progenitor cell populations. Scx is linked with ECM development including the expression of Col1α1 and Tenomodulin in tenocytes.
In conclusion, this study demonstrates one potential strategy to overcome complications with tendon repair due to diabetes, where dACM wrapped tendons resulted in reduced failures, improved mechanical integrity, and greater cell migration to the site of injury in a T2DM animal model. Future work will focus on how placental membranes affect tendon healing on a cellular level and further evaluate the potential mechanisms of action.

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Disclosures

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