**INTRODUCTION:** Cell migration plays an important role in processes such as tissue repair and regeneration. Previous studies have reported that dermal fibroblast migration is influenced by the developmental stage (i.e., fetal versus adult) and the presence of serum factors [1,2]. In addition, collagen expression by dermal fibroblasts has been shown to be age-dependent [3]. However, similar effects on the behavior of tendon fibroblasts are not known. Therefore, the objective of this study was to examine the serum-dependent effects on migration and collagen production in adult and fetal tendon fibroblasts. It was hypothesized that the presence of serum would induce differential cell migration and collagen production in fetal and adult tendon fibroblasts in vitro.

**METHODS:**

**Cell Culture:** Medial tendon specimens from the hind limbs of two time-dated pregnant ewes (80-85 days gestation) and their fetuses were used to isolate tendon fibroblasts. The cells were plated in monolayer culture and maintained in minimum essential medium (MEM) with Earle’s BSS supplemented with 10% fetal bovine serum and antibiotics until confluent. A sterile pipette tip was scraped across the cell monolayer to denude an area of the cells as described by Stenn [4]. Cultures were then rinsed twice with PBS to remove any cellular debris. Next, fibroblasts were cultured in either serum-containing medium (as above) or serum-free, Insulin-Transferrin-Selenium (ITS) (Invitrogen, Carlsbad, CA) medium consisting of MEM with Earle’s BSS, 1X ITS and antibiotics, and allowed to grow for 24 hours.

**Cell Migration:** Images of the cell cultures at 1, 4, 8, 12, 16, and 24 hours were captured (Zeiss Axiovision) and cell migration was analyzed (Zeiss KS300) at the different time points. Five measurements were taken per sample on three different samples for each culture condition.

**RT-PCR:** At 4 hours and 24 hours, total cellular RNA was extracted using the TRIZOL isolation system (Invitrogen). RT-PCR was performed on total RNA extracts using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen). Oligonucleotide primers specific for type I and III collagen, and type I and III collagen were designed based on sequences deposited in GenBank.

**Immunohistochemistry:** At 24 hours, cells were fixed and stained with monoclonal antibodies to types I (Sigma, St. Louis, MO) and III (Chemicon, Temecula, CA) collagen. The cells were visualized using an Alexa Fluor 488-conjugated 2° antibody (Molecular Probes, Eugene, OR). Non-immune controls were performed without primary antibody.

**Statistical Analysis:** Two-way ANOVA with a Fisher’s test was performed to determine the effect of serum and age on cell migration.

**RESULTS:** Fetal tendon fibroblasts migrated faster than adult tendon fibroblasts under both culture conditions (Table 1). After 16 hours, the gap had closed in both fetal and adult tendon fibroblast cultures in serum-containing medium. In contrast, the gap had only closed by approximately 40% in ITS medium after 24 hours. There was a significant difference between the migration rates of the adult tendon fibroblasts compared to the fetal tendon fibroblasts in ITS medium.

**Table 1:** Average Cell Migration Rate (data as mean ± std deviation)

<table>
<thead>
<tr>
<th>Serum</th>
<th>Fetal</th>
<th>ITS</th>
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<tbody>
<tr>
<td></td>
<td>46.5 ± 8.3 μm/hr</td>
<td>24.0 ± 5.2 μm/hr</td>
</tr>
<tr>
<td>Adult</td>
<td>43.7 ± 7.1 μm/hr</td>
<td>18.3 ± 3.7 μm/hr</td>
</tr>
</tbody>
</table>

* denotes sign. diff. between serum and ITS cultures (p < 0.02)
# denotes sign. diff. between fetal and adult cultures (p < 0.02)

However, there was no significant difference in the migration rates between the two cell types in serum-containing medium. There was a significant difference in migration rates for both the adult and the fetal tendon fibroblasts in serum-containing medium versus ITS medium. With respect to collagen production, when grown in serum-containing medium, the adult tendon fibroblasts elaborated more type I collagen than the fetal tendon fibroblasts. However, there was minimal type III collagen produced by both cell types in serum-containing medium. In ITS medium, the fetal tendon fibroblasts showed increased type III collagen production in comparison to adult tendon fibroblasts. The staining was localized to the cells migrating into the denuded area (Fig 1). In the ITS medium, similar levels of type I collagen were found in both the fetal and adult tendon fibroblasts. These results were supported by the RT-PCR gene expression data. In the serum-containing medium, the adult tendon fibroblasts expressed more type I collagen than the fetal tendon fibroblasts, and type III collagen expression was minimal in both cell types. In the ITS medium, the fetal tendon fibroblasts showed increased type III collagen expression compared to those in serum-containing medium, and to adult tendon fibroblasts in ITS medium. Finally, fetal and adult tendon fibroblasts displayed similar type I collagen expression when grown in ITS medium.

**DISCUSSION:** This study suggests that although the migration rate of fetal and adult tendon fibroblasts is serum-dependent, fetal tendon fibroblasts are less affected by the absence of serum than adult tendon fibroblasts. These results are consistent with previous reports of fetal skin fibroblasts exhibiting serum-independent migration behavior in comparison to adult skin fibroblasts [1]. In this study, it was also found that collagen production in tendon fibroblasts was influenced by the presence of serum. Specifically, in the absence of serum (ITS), fetal tendon fibroblasts displayed increased production of type III collagen compared to adult tendon fibroblasts. However, in serum-containing medium, both fetal and adult tendon fibroblasts produced primarily type I collagen, with levels highest in the adult fibroblasts. These results are in agreement with previous studies that report type I collagen production to be enhanced as compared to type III collagen in adult tendon fibroblasts [5]. Similarly, studies have shown type III collagen elaboration to be age-dependent in dermal fibroblasts, with increased production of type III collagen in fetal versus adult dermal fibroblasts [6]. The altered collagen production and migration rates we observed in ITS medium could be a result of the lack of serum factors, such as platelet-derived growth factor, which have been shown to stimulate cell migration and type I collagen synthesis [7,8]. Since cell migration and collagen secretion have been associated with tendon wound healing, these findings may provide insight into the cellular mechanisms involved in fetal and adult tendon wound healing. Future studies will investigate proteoglycan expression by tendon fibroblasts and examine the behavior of these cells in 3-D constructs.

**REFERENCES:**


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