Evaluation of an Amniotic Suspension Allograft for Tendon Repair in Vitro

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Statement of Purpose

Placental tissues were first used for wound and burn applications in the early 1900s; further research has shown that these membranes also contain a number of growth factors and cytokines beneficial to tendon healing, including FGF, IGF-I, PDGF, TGF-β, and TIMPs. The purpose of this study was to investigate growth factors and cytokines important to tendon repair and potential mechanisms of action using a commercially-available amniotic suspension allograft (ASA) consisting of cells from the amniotic fluid and amniotic membrane particulate.

Methodology & Hypothesis

Growth factors from ASA (n=20 donors) were measured using a quantitative multiplex ELISA. Commercially-available human adult tenocytes from three male donors ages 61-81 were used for experiments. Tenocyte proliferation was evaluated by adding conditioned media (CM) made from ASA at concentrations of 50%, 25%, 10% (v/v) CM and measuring cell proliferation using AlamarBlue at days 3, 7, 10, and 14 (n=12 per group). Tenocyte migration was evaluated using standard Boyden chamber assays; ASA CM was added at concentrations of 50%, 25%, 10%, 1% (v/v) and assessed following 24 hours of migration (n=24 per group). Gene expression of ECM components, including Col1A1, Col3A1, elastin (ELN), decorin (DCN), tenascin C (TNC), and VEGF, were quantified in tenocytes with and without ASA CM. Cells were collected, then RNA was isolated and converted to cDNA using reverse transcription. TaqMan probes were used to evaluate fold-change expression compared to the GAPDH housekeeping gene.

Our overall hypothesis was that placental-derived products stimulate tendon repair. To assess this, we evaluated the effects of an ASA on tenocyte proliferation, migration, and ECM gene expression.

Literature Review

Placental-derived membranes have been used historically for severe wound and burn cases; after application of the membranes, patients had less infection and trauma, more robust healing, and decreased pain[1,2]. Amniotic membranes contain a number of molecules, including IL-1Ra and TIMPs, which can promote an anti-inflammatory environment[3,4], as well as IGF-I and TGF-β, which support tendon healing processes[5,6]. Presently, placental-derived membrane are becoming more widespread for utility in orthopaedic treatments[7]. One alternative to intact membrane grafts is the use of an injectable ASA, which combines the benefit of amniotic membrane and cells

Literature Review

from the amniotic fluid[8]. Clinical studies in various applications have shown reduced pain, improved mobility, and higher quality of life after treatment with ASA[8-9], suggesting a potential benefit in the use of ASA in tendon repair applications.

Results

Regenerative	Anti-Inflammatory
bFGF	IL-6
aFGF	IL-1Ra
PDGF-AA	TIMP-1
PDGF-BB	TIMP-2
TGF-β3	TIMP-4
TGF-β1	
IGF-I	

Table 1: Growth factors present in ASA relevant to tendon healing. Testing results from 20 human donors.

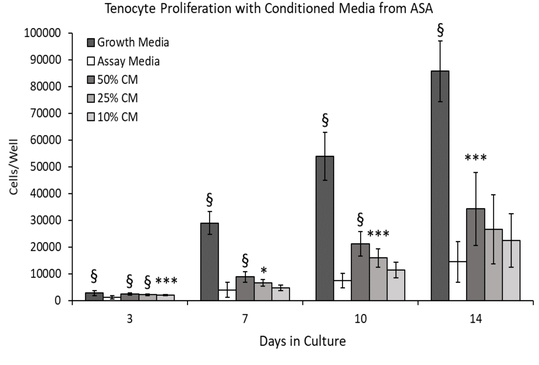

Figure 1: Tenocyte proliferation with ASA conditioned media. Mean ± standard deviation reported; n=12 per group. * denotes p<0.05; *** denotes p<0.001, § denotes p<0.0001 compared to assay media control.

Results

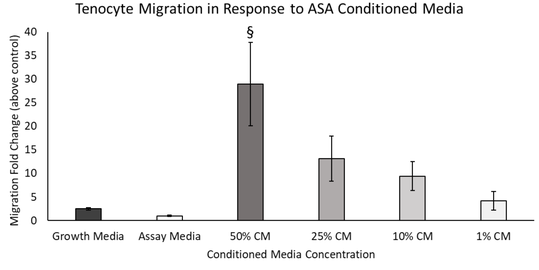

Figure 2: Tenocyte migration with ASA CM. Mean ± standard deviation reported; n=24 per group. § denotes p<0.0001 compared to assay media controls.

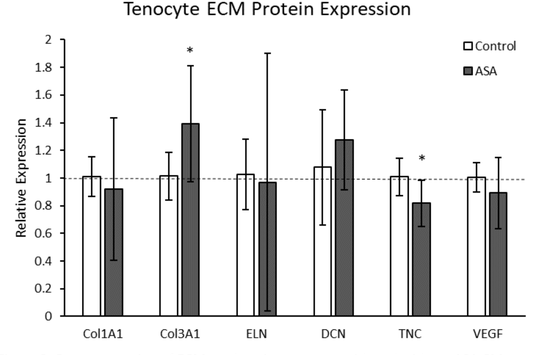

Figure 3: Gene expression of ECM markers in tenocytes with and without ASA CM. Mean ± standard deviation reported; n=9 per group.

Analysis & Discussion

ASA contained physiologically-relevant levels of growth factors, including aFGF, bFGF, IGF-I, IL-1Ra, IL-6, PDGF-BB, TGF-β1, TGF-β3, TIMP-1, TIMP-2, and TIMP-4 (Table 1). These factors have been implicated in tendon repair in preclinical and clinical studies[10-12]. Furthermore, when examining the effect of ASA CM on tenocyte proliferation, there was a significant increase

Analysis & Discussion

in the 50% CM compared to assay controls at all four time points (days 3, 7, 10: p<0.0001; day 14: p<0.001) (Figure 2). 25% CM was significantly increased at day 3 (p<0.001), day 7 (p<0.05), and day 10 (p<0.001), while 10% CM was only significant at day 3 (p<0.001). We hypothesize this response is due to the presence of FGF, PDGF-BB, and IGF-I in the ASA[3]. When examining migration, 50% CM from ASA resulted in a significant increase (27-fold, p<0.0001) compared to assay media controls (Figure 3). ASA contains both bFGF and TGF-β3, which have promoted migration in previous preclinical studies[13,14].

Gene expression of ECM proteins was examined in tenocytes with and without ASA CM. Col3A1 expression was significantly increased with ASA CM, while tenascin C (TNC) was significantly decreased compared to assay media controls. Previous studies have shown that TGF-β3 promotes Col3A1 expression, which is indicative of a repair and remodeling response by tenocytes[14-15]. Tenascin C expression has been linked to mechanical stress and inflammation; the decrease in gene expression with ASA CM may imply treatment results in a more anti-inflammatory environment[16].

ASA contains growth factors and cytokines relevant to tendon repair, which play a role in promoting proliferation, migration, and ECM deposition in human tenocytes.

References

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Disclosures

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