Evaluation of Two Distinct Placental-Derived Membranes and Their Effect on Tendon Repair Mechanisms

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Statement of Purpose

Placental-derived membranes have been traditionally utilized in an adhesion barrier application; however, these membranes are known to contain growth factors critical to robust healing of injured tendons, including aFGF, bFGF, IGF-1, PDGF, and TGF-β1. The purpose of this study was to evaluate levels of these important factors and investigate potential mechanisms of action in tendon repair with two placental-derived membranes: a fresh hypothermically-stored amniotic membrane (HSAM) and a dehydrated human amnion/chorion membrane (dACM).

Methodology & Hypothesis

Growth factors from HSAM and dACM (n=7-9 donors per group) and what was released from those grafts over 3-5 days (releasate, n=4 donors per group) were measured using a quantitative multiplex ELISA. Commercially-available human adult tenocytes from three male donors ages 61-81 were used for all cell experiments. For tenocyte proliferation, releasate from both HSAM and dACM were added at 50%, 25%, and 10% (v/v) concentrations over 14 days and proliferation measured using AlamarBlue (n=12 per group). For tenocyte migration, standard Boyden chamber assays were conducted; specifically, releasate from HSAM and dACM 50%, 25%, 10% (v/v) concentrations were added to wells and migration was evaluated after 24 hours (n=24 per group). To evaluate the effect of the ECM itself, we evaluated the attachment and migration of tenocytes into HSAM and dACM membranes. To do this, cells were cultured directly on grafts for 4 weeks and attachment and migration was evaluated using H&E staining. Interactions in the context of inflammation were evaluated by measuring production of TGF-β1 and MMP-1 by tenocytes in response to inflammation with and without dACM and HSAM by using ELISAs.

Our overall hypothesis is that placental products promote tendon repair; to evaluate this, we used two distinct placental-derived products (HSAM and dACM) and evaluated their interactions with tenocytes by measuring tenocyte responses including proliferation, migration and gene expression.

Literature Review

Placental-derived tissues have a rich history for use in wound healing applications[1]; recently, utility for orthopaedic ailments has become popular[2,3]. Preclinical studies have shown improved structural and mechanical repair, with quicker healing times[4,5]. Early clinical studies have shown reduced pain and improved function in the affected limb following treatment[6-8]. Human placental tissues are rich in growth factors, cytokines, ECM, and MSCs; however, allograft composition depends on collection and processing techniques[9,10].

Results

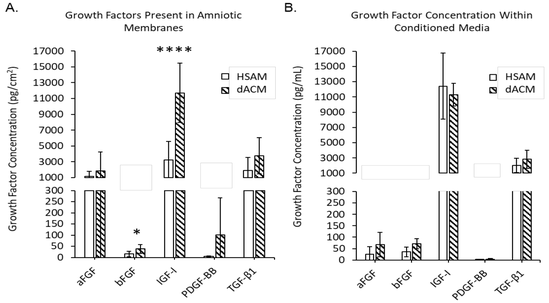

Figure 1: Growth factors present in both (A) HSAM and dACM and (B) growth factors released from HSAM and dACM into conditioned media. Mean ± standard deviation reported; n=4-9 per group. * denotes p<0.05; **** denotes p<0.0001.

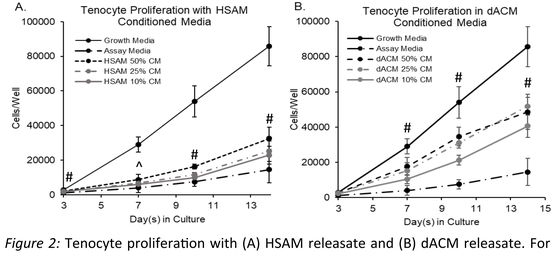

Figure 2: Tenocyte proliferation with (A) HSAM releasate and (B) dACM releasate. For both figures, mean ± standard deviation reported; n=12 per group. # denotes all groups significant to assay media controls (p<0.01); ^ denotes all significant except 10% CM.

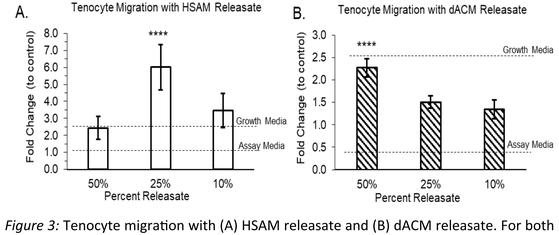

Figure 3: Tenocyte migration with (A) HSAM releasate and (B) dACM releasate. For both figures, mean ± standard deviation reported; n=24 per group. **** denotes p<0.0001 compared to assay media controls. Dotted lines indicate migration levels for assay media (negative control) and growth media (positive control).

Results

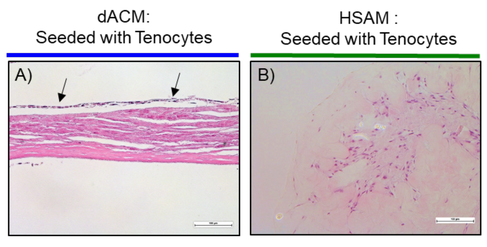

Figure 4: Representative H&E images of tenocytes co-cultured with (A) dACM and (B) HSAM for 4 weeks. Scale bar indicates 100µm.

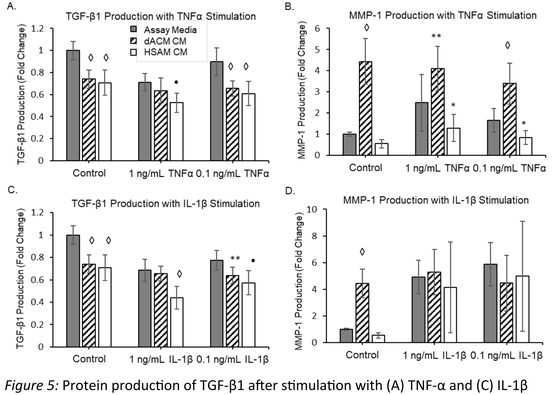

Figure 5: Protein production of TGF-β1 after stimulation with (A) TNF-α and (C) IL-1β and MMP-1 after stimulation with (B) TNF-α and (D) IL-1β. Mean ± standard deviation reported; n=9 for all groups. * denotes p<0.05; ** denotes p<0.01, ● denotes p<0.001; ◊ denotes p<0.0001 compared to assay media control; * and ● indicate significance between dACM and HSAM groups.

Analysis & Discussion

HSAM and dACM contained physiologically-relevant levels of growth factors (Figure 1A) thought to promote tendon healing, including IGF-I, PDGF-BB, aFGF, and bFGF[11]. Additionally, we evaluated release of growth factors from both dACM and HSAM and found comparable levels (Figure 1B). Releasate from both HSAM and dACM were found to significantly increase tenocyte proliferation at day 3 for all releasate percentages (p<0.01) (Figure 2A, 2B);

Analysis & Discussion

dACM releasate continued to be significant to day 14 (p<0.0001). HSAM releasate was also significant to day 14 (p<0.01) except for the day 7 10% group. Migration of tenocytes with HSAM and dACM releasate showed a significant fold-change increase compared to assay media at 25% and 50%, respectively (p<0.0001) (Figure 3A, 3B). The presence of IGF-I, TGF-β1, and FGF have been shown to increase the proliferation and migration of tenocytes[11]. When evaluating ECM interactions, tenocytes attached, but did not migrate into the dACM graft (Figure 4A); however, the HSAM ECM allowed for extensive tenocyte attachment and migration (Figure 4B). Fresh membrane (HSAM) preserves the ECM, while dehydrated membrane (dACM) results in a compressed ECM structure[10].

When tenocytes were exposed to inflammation, exposure to HSAM and dACM releasate resulted in a significant drop in TGF-β1 production, which could translate to a reduction in scar and adhesion formation clinically[12] (Figure 5A, 5C). Interestingly, when tenocytes were stimulated with TNF-α, HSAM releasate resulted in reduced MMP-1 production and dACM resulted in increased MMP-1 production (Figure 5B, 5D); these results could be due to protease inhibitor levels in each placental-derived product[13].

HSAM and dACM contain and release growth factors and cytokines that may promote tendon repair. Additionally, we evaluated the effects of processed ECM on tendon responses. While both grafts resulted in increased tenocyte proliferation, migration and gene expression, there were discreet differences in these responses. In sum, we found that these membranes may work in different ways and have varying tenocyte responses in promoting repair.

References

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Disclosures

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