Insights into the Proteomic Composition of Processed Amniotic Membrane Bioscaffolds for Orthopedic Indications

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INTRODUCTION: Amniotic membrane-derived (AM) bioscaffolds have been used extensively to improve wound healing outcomes for decades. Offering abundant supply and hosting a reservoir of regenerative protein cues, these tissues exhibit anti-fibrotic and anti-inflammatory properties that have demonstrated repair of various tissues. Owing to these characteristics, AM bioscaffolds have proven effective as surgical barriers in spinal fusion procedures. Processing tissues for clinical applications is necessary to prevent infection and host rejection by removing cellular debris, leaving behind a protein extracellular matrix (ECM) bioscaffold that influences host cellular response. However as tissue processing methods strongly influence resultant bioscaffold properties, the ECM proteomic composition of amniotic tissues underlying host response throughout the literature remain poorly described. The **purpose of this study** was to determine the ECM proteomic composition of processed amniotic wrap (AW) materials, yielding well-defined bioscaffolds while elucidating their anti-fibrotic and anti-inflammatory properties. In this study, two AW materials were compared by either processing as dry (pAW-Dry) or hydrated (pAW-Wet) formulations, as each has respective surgical applications and appeal. Our **hypothesis** was that the difference in tissue processing methods would affect the ECM properties between pAW-Dry and pAW-Wet, offering molecular insight into how the AM improves patient outcomes.

METHODS: Human amniotic membrane was recovered from consenting cesarean sections and processed using proprietary methods to produce wet (pAW-Wet; n=3) or dry (pAW-Dry; n=3) formats (AlloSource, Centennial, CO). Fresh, unprocessed AM was provided as a control group (n=3) for molecular comparison. Unprocessed AM, pAW-Dry, and pAW-Wet were submitted to Omix Technologies for untargeted tissue proteomics using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described in McCabe 2021. Briefly, tissues were subjected to ECM-focused solubilization, trypsin digestion, followed by LC-MS/MS using Evosep One coupled to timsTOF Pro. Raw spectra were searched in MSFragger, and proteins are expressed here as relative signal intensity. Protein levels were determined to be statistically significant as $P < 0.05$ using Wilcoxon signed-rank test or non-parametric Welch's ANOVA with Tukey-HSD post-hoc test as appropriate. Enrichment for biological processes was performed using Metascape.

RESULTS SECTION: Unsupervised hierarchical clustering of significantly different proteins among Unprocessed AM, pAW-Dry, and pAW-Wet (n=3 each) using ANOVA ($Q < 0.05$) revealed the loss and concentration of proteins in AW following processing (Fig. 1A). Both pAW-Dry and pAW-Wet had losses in cornified envelope proteins (S100A10, keratins, envoplakin) and proteins involved in elastin microfibril assembly (MFAP2), as compared to Unprocessed AM. Tissue processing significantly enriched AW for proteins involved in lysosomal activity, degranulation, and cell stress. There were also elevations in protein glycosylation and cytoskeletal proteins in both AW formulations. As pAW-Dry and pAW-Wet are used for specific clinical applications, we next determined proteomics composition differences between these. pAW-Dry showed significant enrichment of proteins involved in the cytoskeleton, metabolism, and wound healing, while pAW-Wet enriched for angiogenesis, ECM organization, and inflammation (immunoglobulins) (Fig. 1B). Interestingly, pAW-Dry had significant elevations in Microfibril Associated Protein (MFAP2) which is involved in ECM organization and assembly of larger ECM fibers, as well as plectin (PLEC) which has various roles in wound healing (Fig. 1C). pAW-Wet had elevations in minor and major collagens (COL1A2) which provide structural support, as well as periostin (POSTN) which is involved in tissue development and osteogenesis.

DISCUSSION: Here we provide quantitative insight into the structural foundation of AW, a crucial step for understanding their role in tissue healing and regeneration. Processing AM alters concentrations of proteins with known roles in fibrosis (SEPTIN2, COLGALT1, THBS1), inflammation (degranulation), and tissue remodeling (lysosomes, glycosylations), all biological processes which affect repair outcomes. Further, pAW-Dry and pAW-Wet both display unique proteomics signatures of metabolic modulation and wound healing, and ECM organization, respectively. Ongoing and future work is targeted towards understanding how retained proteins across AW products can be enriched to improve patient outcomes following surgery.

SIGNIFICANCE/CLINICAL RELEVANCE: Amniotic membrane-based materials are emerging bioscaffolds with evidence of anti-fibrotic and anti-inflammatory properties. Careful understanding of how various processing techniques influence the composition of these materials, and ultimate therapeutic value, is crucial towards their successful adoption in orthopaedic indications where anti-fibrosis and anti-inflammation is desired

IMAGES AND TABLES:

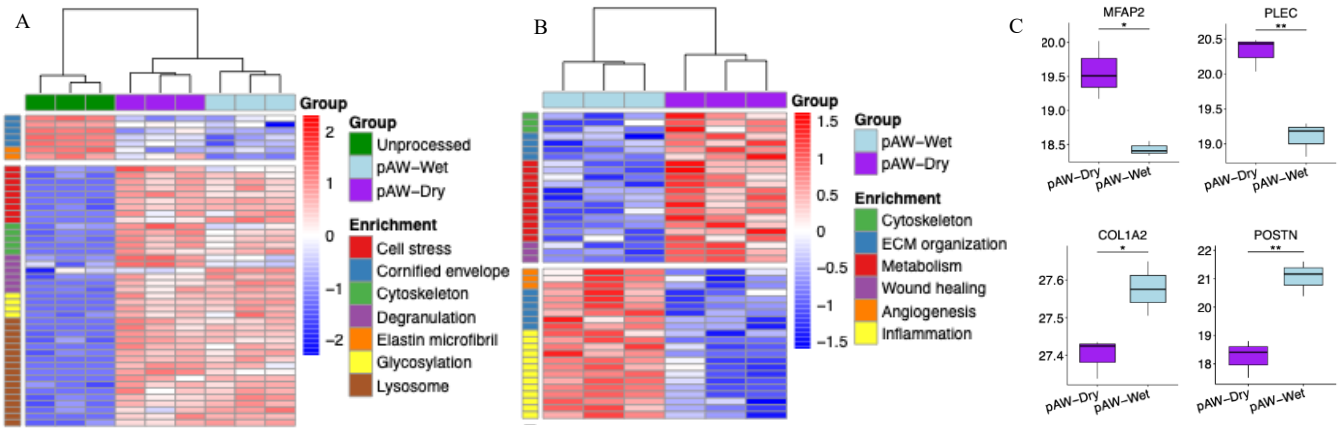

Figure 1: Significantly different proteins among A) Unprocessed AM, pAW-Wet, and pAW-Dry; and B) pAW-Wet vs. pAW-Dry. C) Boxplots of proteins of interest. *$P < 0.05$, **$P < 0.005$