Drying Method and Irradiation Effects on Birth Tissue Regulatory Proteins

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Background: Birth tissue membranes have been used clinically for approximately 100 years because of their healing properties. Proper processing methods are crucial to preserve the extracellular matrix and signaling molecules that are known to promote tissue healing and regeneration. Dehydration (e.g., air drying) is a common preservation method for birth tissue that removes liquid water from the tissue. Lyophilization (also known as freeze-drying) is another form of tissue preservation that often is preferable to dehydration since lyophilization prevents protein denaturation and other similar physical changes to biomolecules. Gamma radiation is a terminal sterilization method where the ionizing radiation inactivates microorganisms through chemical alterations to their DNA and proteins. Ionizing radiation may alter the tissue and impact the proteins or other biomolecules. In this study we determined the effects of different drying methods (dehydration vs. lyophilization) and radiation on growth factors using enzyme linked immunosorbent assays (ELISA).

Hypothesis: Dehydration and radiation will have a negative effect on growth factors as detected by ELISA.

Method: Physically cleaned birth tissue membranes from three donors (either amnion alone or amnion with attached chorion) were subjected to either dehydration or lyophilization. The dried tissues were gamma irradiated using a proprietary radiation process or were not irradiated. The tissues were extracted using RIPA buffer. The extracts were tested for TIMP1 (Tissue Inhibitor of Metalloproteinase 1) and β-FGF (beta Fibroblast Growth Factor) using ELISA. Cleaned tissues from the same donors, not subjected to drying or radiation, were evaluated as controls.

Results: LifeLink's proprietary lyophilization process preserved regulatory proteins (TIMP1 and β-FGF) in a similar range to those found in the control group for amnion/chorion membranes. The protein levels were maintained to a higher degree for lyophilization compared to dehydration for amnion and amnion/chorion. LifeLink's proprietary gamma irradiation method did not appear to impact protein levels. Additionally, amnion/chorion has considerably higher protein levels than amnion alone.

ANOVA general linear model was used to determine if the factors of drying method (dehydration, lyophilization), radiation method (radiated, not radiated) and membrane layers (amnion alone, amnion/chorion) showed a statistical difference for the dried tissues. Individual ANOVAs were run for TIMP-1 and β-FGF; $p<0.05$ was considered statistically significant. TIMP-1 and β-FGF levels for lyophilized tissues were statistically higher than for dehydrated tissues. There was no statistical difference between the radiated and non-irradiated tissues for both growth factors. Amnion/chorion has statistically significantly higher TIMP-1 and β-FGF levels than amnion alone.

Conclusion: This study demonstrates that the proprietary lyophilization process maintains the regulatory proteins to a higher degree than dehydration, and the proprietary radiation process had no significant effect on these proteins.

Drying Method and Irradiation Effects on Birth Tissue Regulatory Proteins

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Background

Birth tissue membranes have been used clinically for approximately 100 years because of their healing properties. Proper processing methods are crucial to preserve the extracellular matrix and signaling molecules that are known to promote tissue healing and regeneration. Dehydration (e.g., air drying) is a common preservation method for birth tissue that removes liquid water from the tissue. Lyophilization (also known as freeze-drying) is another form of tissue preservation that often is preferable to dehydration since lyophilization prevents protein denaturation and other similar physical changes to biomolecules. Gamma radiation is a terminal sterilization method where the ionizing radiation inactivates microorganisms through chemical alterations to their DNA and proteins. Ionizing radiation may alter the tissue and impact the proteins or other biomolecules. In this study we determined the effects of different drying methods (dehydration vs. lyophilization) and radiation on growth factors using enzyme linked immunosorbent assays (ELISA).

Hypothesis

Dehydration and radiation will have a negative effect on growth factors as detected by ELISA.

Method

Physically cleaned birth tissue membranes from three donors (either amnion alone or amnion with attached chorion) were subjected to either dehydration or lyophilization. The dried tissues were gamma irradiated using a proprietary radiation process or were not irradiated. The tissues were extracted using RIPA buffer. The extracts were tested for TIMP1 (Tissue Inhibitor of Metalloproteinase 1) and β-FGF (beta Fibroblast Growth Factor) using ELISA. Cleaned tissues from the same donors, not subjected to drying or radiation, were evaluated as controls.

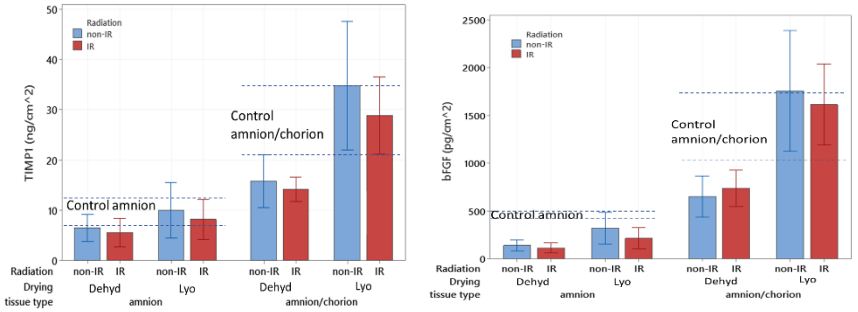

Figure 1. Effect of tissue type (amnion vs amnion/chorion), drying method (dehydration vs. lyophilization) and irradiation (non-IR vs. IR) on TIMP-1 and β-FGF levels measured by ELISA. Top shows TIMP-1 results and bottom shows β-FGF results.

Table 1. Statistical analysis using analysis of variance (ANOVA) general linear model by Minitab version 21.

Tissue or process related factor	Levels	Regulatory Protein	P-Value	Conclusion
Membrane layers	amnion alone vs. amnion/chorion	β-FGF	0.000	amnion+chorion contains significantly higher regulatory protein levels than amnion alone for model proteins studied.
		TIMP-1	0.000	
Drying method	dehydration vs. Lyophilization	β-FGF	0.000	Lyophilization preserves the model proteins studied better than dehydration.
		TIMP-1	0.000	
Radiation sterilization	radiated vs. not radiated	β-FGF	0.637	The radiation process evaluated had no significant impact on the model proteins studied.
		TIMP-1	0.217	

Results

Figure 1 shows the ELISA results. LifeLink's proprietary lyophilization process preserved regulatory proteins (TIMP1 and β-FGF) in a similar range to those found in the control group for amnion/chorion membranes. The protein levels were maintained to a higher degree for lyophilization compared to dehydration for amnion and amnion/chorion. LifeLink's proprietary gamma irradiation method did not appear to impact protein levels. Additionally, amnion/chorion has considerably higher protein levels than amnion alone.

Table 1 shows the statistical analysis details. ANOVA general linear model was used to determine if the factors of drying method (dehydration, lyophilization), radiation method (radiated, not radiated) and membrane layers (amnion alone, amnion/chorion) showed a statistical difference for the dried tissues. Individual ANOVAs were run for TIMP-1 and β-FGF; $p<0.05$ was considered statistically significant. TIMP-1 and β-FGF levels for lyophilized tissues were statistically higher than for dehydrated tissues. There was no statistical difference between the radiated and non-irradiated tissues for both growth factors. Amnion/chorion has statistically significantly higher TIMP-1 and β-FGF levels than amnion alone.

Conclusion

This study demonstrates that the proprietary lyophilization process maintains the regulatory proteins to a higher degree than dehydration, and the proprietary radiation process had no significant effect on these proteins.

Future Work

RegeneLink™ (Lyophilized irradiated human amnion-chorion membrane by LifeLink Tissue Bank) will be launched based on these and other data on August 2023.