

PLACENTAL MEMBRANE COVERING PROCESSING: MAINTAINING CHARACTERISTICS OF SOURCE TISSUE WHILE MAXIMIZING NORMALIZATION, QUALITY AND SAFETY.

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INTRODUCTION

Research on the therapeutic potential of perinatal birth tissue for wound care in a clinical setting has increased exponentially as clinicians, scientists, researchers, and manufacturers continue to innovate and utilize this tissue for its protective properties. In order to effectively measure outcomes, several factors must be considered. Birth tissue is dynamic and varies widely from one donation to the next. Standardization (reduction of variations from each lot in birth tissue) of a final product is critical to clinical utility. Without proper processing techniques, it is not possible to address this variance and ensure product quality, product safety and product consistency.

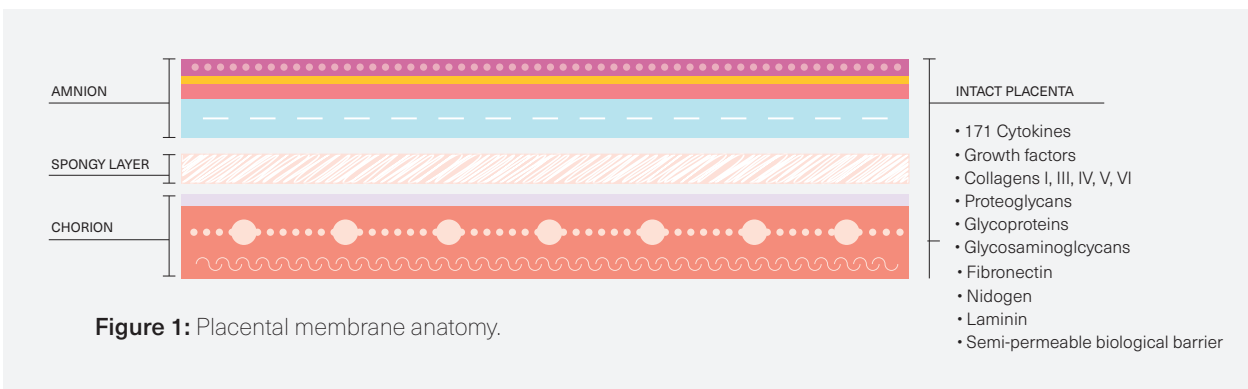
Predictive Biotech's human cell and tissue product (HCT/P), AmnioBind™, is a dehydrated, intact placental membrane that preserves the naturally occurring cytokines, growth factors, comprehensive collagen matrix, glycoconjugates, and glycosaminoglycans. It is derived from the placental membranes from donated, full-term birth tissue. Equipped with state-of-the-art analytical equipment to ensure the safety and viability of its allografts, Predictive Biotech's laboratory and proprietary processes maintain the tissue's biological components with minimal degradation of native proteins.

BACKGROUND

Placental Membrane

Anatomically, the human placental membranes surround the fetus and separate it from maternal tissue. The membrane provides elasticity and strength to hold, cushion, and protect a developing fetus and acts as a critical barrier, preventing a mixing of maternal and fetal blood and the transfer of pathogens, while allowing key nutrients to pass to the fetus. The fetal membranes consist of two layers: the outer chorion which is in contact with the uterine wall, and the amnion (amniotic membrane) which is avascular, is on the fetal side and is in contact with amniotic fluid. The amniotic membrane's basal surface lies on top of the chorion, the outer most layer of the placental membrane.¹ The amniotic membrane and the chorion are not bonded and remain separable even after delivery.² (Figure 1) Human placental membranes are thin membranes that are mostly comprised of collagen, laminin, and fibronectin.³ Their composition includes (from amniotic layer to chorionic layer):

- Epithelium
- Basement membrane composed of collagen (III, IV, V), laminin, fibronectin, and nidogen
- Compact layer composed of collagen (I, III, V, VI) and fibronectin
- Fibroblast layer composed of collagen (I, III, VI), nidogen, laminin, and fibronectin
- Intermediate (spongy) layer composed of collagen (types I, III, IV) and proteoglycans
- Chorion layer composed of a reticular layer (collagens I-IV, elastin), a pseudobasement membrane, and trophoblast layer that contacts the maternal decidua⁴



Function of Amniotic Membrane

The amniotic membrane is biological structure devoid of nerves, muscles, or lymph vessels. It receives nutrients and oxygen from the chorionic fluid and transfers it across the membrane to the fetal surface vessels.⁵ As it protects the growing fetus from the pressures of surrounding structures, it also facilitates metabolic functions, such as the transport of water and soluble materials, and produces important biological factors, such as cytokines and growth factors.⁶

AmnioBind Product Description

AmnioBind™ is a dehydrated intact placental membrane that preserves the naturally occurring cytokines, growth factors, collagens, laminins, fibronectins, nidogens, elastins, glycoconjugates, and glycosaminoglycans.

Our in-house propriety process has allowed us to preserve the placental membrane with little to no degradation of the naturally occurring proteins during the dehydration and terminal sterilization process.

Upon the application of AmnioBind™ the cytokines, growth factors, as well as the structural proteins are returned to a near-native state which provides an ideal protective barrier for wounds. By preserving all layers of the placental membrane, AmnioBind™ is a protective barrier with exceptional structural integrity.

AMNIOBIND PROCESSING

Once the tissue has been received by our laboratory and thoroughly cleaned the placental membrane is then processed by our lab technicians. An important note to consider is that there is NO separation of the Amnion and Chorion layers, which helps to preserve the intermediate layer that contains naturally present cytokines, growth factors, collagens, and glycoproteins. We do not have to rely on artificial binding or fusion of separated layers. By not scraping and separating the membranes, we are able to preserve the structural components found within the intermediate layer.

Our processing removes any cellular remnants and sterilizes the placental membrane without loss of the important components crucial to the function of the membrane as a biological covering. To maintain the natural characteristics of the placental membrane, no chemicals or antibiotics are used. This ensures that there is no risk of selecting antibiotic resistant bacteria and no chemical dehydration means no risk of residual irritants or chemical denaturation of protein. AmnioBind™ is never frozen and completely free of preservatives which allows us to maintain its shape and protect the proteins and structures that provide the characteristics crucial to the membrane function as a biological covering.

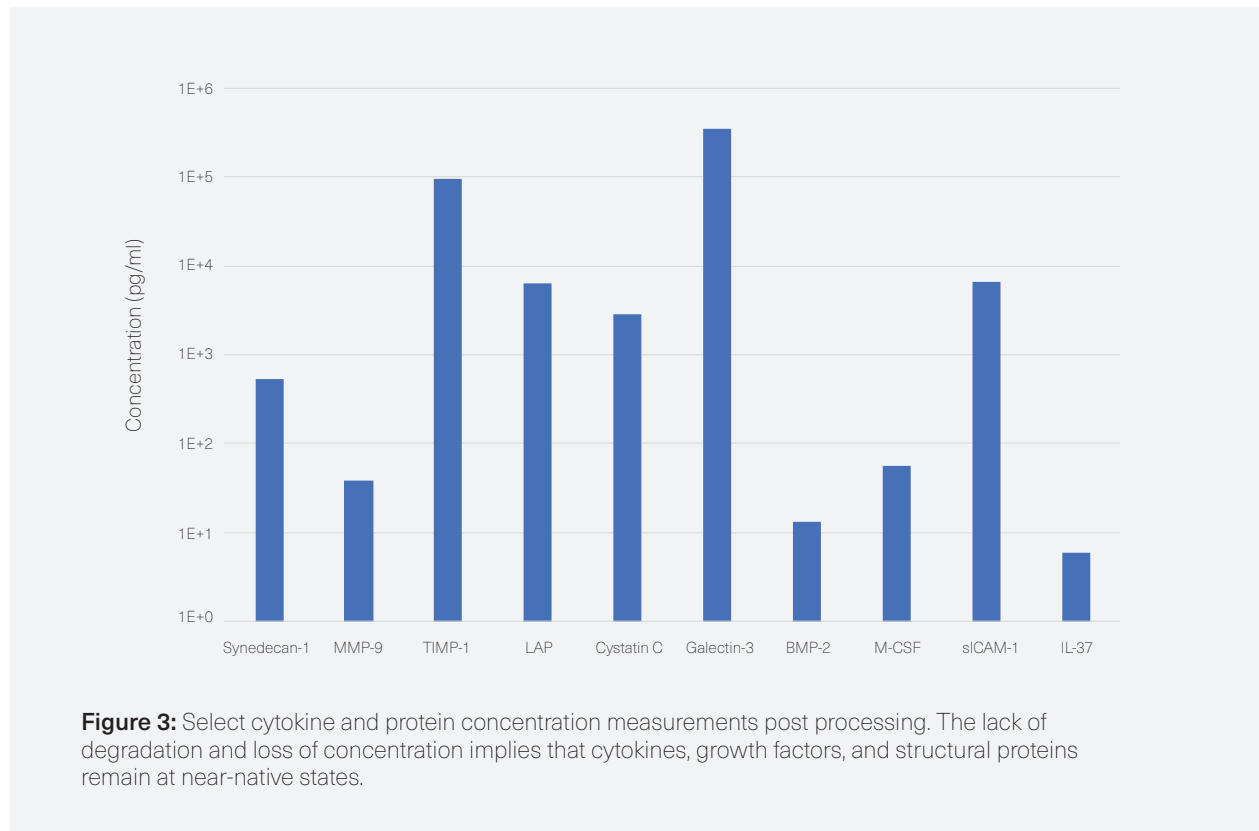
CYTOKINES AND GROWTH FACTORS

Cytokines are a broad group of small cellular proteins involved in biological processes such as tissue remodeling. Birth tissue contains a rich and diverse population of cytokines, underscoring the dynamic nature of the tissue. These often-overlooked proteins are critical tissue components that must be accounted for and maintained in the manufacturing process of donated tissue. Predictive Biotech's amniotic membrane products were analyzed by RayBiotech using a Quantibody[®] array-based multiplex ELISA system for simultaneous quantitative measurement of various cytokines, growth factors, proteases, soluble receptors, and other proteins. (Figure 2)

Test	Description	Avg pg/mL	Test	Description	Avg pg/mL	Test	Description	Avg pg/mL
CYT5	Ferritin	328,296.3	CYT6	Galectin-3	5,106.5	CYT8	IGFBP-5	1,645.8
CYT7	Fetuin A	273,161.9	CYT4	Resistin	4,764.7	CYT4	Siglec-5	1,644.3
CYT8	DPPIV	65,701.7	CYT8	IL-5 Ra	4,646.6	CYT10	Galectin-9	1,633.1
CYT5	Adiponectin	63,751.6	CYT5	IGF-1R	4,573.0	INF3	IL-1ra	881.8
CYT6	APRIL	61,546.3	REC1	PECAM-1	2,114.3	INF3	TNF RI	868.7
CYT9	Angiotensinogen	59,503.0	CYT10	Pentraxin 3	4,505.5	CYT9	EMMPRIN	1,593.8
INF3	TIMP-2	6,723.6	CYT5	MMP-9	4,483.4	CYT6	FAP	1,533.6
CYT7	TSP-1	55,739.1	CYT9	FLRG	4,412.1	CYT6	Nephrilysin	1,471.1
CYT9	Periostin	39,157.6	CYT10	ADAM8	4,261.5	CYT6	DcR3	1,469.9
CYT10	CD84	38,858.4	CYT4	Galectin-7	4,085.8	CYT6	WIF-1	1,444.4
CYT5	ANGPTL4	36,877.6	CYT10	CD48	4,060.9	CYT8	MMP-7	1,382.9
CYT4	VEGF R1	26,987.3	CYT9	Persephin	3,964.0	CYT7	WISP-1	1,374.7
CYT4	PAI-1	24,160.0	CYT9	Tie-1	3,784.1	CYT4	E-Cadherin	1,345.8
CYT9	Follistatin-like 1	22,311.2	CYT9	B7-H1	3,749.6	CYT6	IGF-2R	1,338.3
CYT5	MMP-2	14,746.3	CYT5	CA125	3,744.8	REC1	MICB	717.6
CYT7	VE-Cadherin	14,646.4	REC1	ALCAM	1,670.0	CYT4	IL-13 R2	1,289.6
CYT9	Thrombospondin-5	13,237.4	CYT6	Thrombomodulin	3,636.3	CYT4	ICAM-2	1,254.7
CYT7	Albumin	12,679.5	CYT7	Dkk-3	3,535.4	CYT8	CHI3L1	1,235.2
CYT5	hCGb	12,458.3	CYT7	Decorin	3,231.8	CYT7	TACI	1,205.2
CYT7	CD163	11,841.0	CYT8	Cathepsin B	3,143.8	CYT9	IL-17E	1,204.1
CYT9	L1CAM-2	10,970.3	CYT4	DKK-1	3,056.9	CYT8	PSMA	1,177.2
CYT9	DNAM-1	10,868.1	REC1	Trappin-2	1,572.2	CYT10	Galectin-2	1,173.9
CYT9	LRP-6	10,646.5	CYT6	sFRP-3	2,967.7	CYT7	Clusterin	1,172.6
CYT10	Pref-1	10,552.1	CYT7	TRANCE	2,831.6	CYT7	ANG-4	1,171.7
CYT9	ADAMTS13	9,159.8	REC1	CD14	1,493.7	CYT10	DR3	1,140.3
CYT7	Furin	8,912.8	CYT4	gp130	2,828.0	CYT5	NCAM-1	1,123.8
INF3	ICAM-1	3,891.9	CYT9	Granulysin	2,669.4	REC1	uPAR	647.2
CYT5	Adipsin	8,744.1	CYT6	Cathepsin L	2,649.1	REC1	RAGE	645.3
CYT9	CD6	8,674.3	CYT10	CEACAM-5	2,611.2	CYT5	OSM	1,115.8
CYT4	DAN	8,557.6	CYT10	SP-D	2,610.1	REC1	Lipocalin-2	637.8
CYT10	CD155	8,395.7	CYT5	MMP-1	2,608.5	CYT7	RANK	1,093.6
CYT7	Syndecan-1	8,164.1	CYT4	Fcg RIIBC	2,583.2	CYT5	AFP	1,078.3
INF3	TIMP-1	3,475.2	CYT10	ICOS	2,402.5	CYT9	Aggrecan	1,067.4
CYT7	RBP4	8,131.9	CYT10	CD58	2,400.2	CYT7	LAG-3	1,009.7
CYT6	Chemerin	7,962.2	CYT9	CNTF	2,333.4	CYT5	CA15-3	1,002.2
CYT5	Nidogen-1	7,708.3	CYT7	ACE-2	2,329.2	CYT5	MMP-13	991.5
CYT7	CA19-9	7,676.8	CYT5	CRP	2,328.5	REC1	VCAM-1	592.8
CYT5	NSE	7,658.1	CYT5	Procalcitonin	2,305.9	CYT10	ULBP-1	972.7
CYT4	Angiostatin	7,618.5	CYT8	Leptin R	2,298.4	CYT9	BMPR-II	912.9
CYT9	LRIG3	7,476.4	CYT7	AMICA	2,013.3	CYT5	Thyroglobulin	837.6
CYT6	CD97	7,205.5	CYT10	B7-H3	1,934.9	CYT6	IFNab R2	818.3
CYT8	biG-H3	7,075.2	CYT9	Nectin-4	1,890.4	CYT6	Legumain	755.9
CYT8	IL-10 Ra	6,457.2	CYT10	Cadherin-4	1,884.6	CYT8	IL-1 F10	747.5
CYT5	Prolactin	6,344.5	CYT7	DLL1	1,874.3	CYT4	FAS L	737.9
CYT10	Siglec-10	6,296.7	CYT8	IL-1 F7	1,824.1	CYT8	SIGIRR	735.0
CYT9	BMPR-IA	6,058.5	CYT6	CD200	1,807.9	CYT6	HGF R	709.2
CYT9	Fractalkine	6,023.8	CYT7	IL-17B R	1,793.7	REC1	LYVE-1	507.7
CYT6	Transferrin	5,737.8	CYT10	Cystatin B	1,724.8	CYT9	ULBP-2	700.2
CYT6	Serpin A4	5,533.8	CYT4	IL-13 R1	1,704.6	CYT6	C5a	695.6
CYT10	Desmoglein 2	5,377.1	CYT7	CXCL14	1,665.5	CYT6	IL-1 R6	689.0

Figure 2: Representative list of 150 of the 171 present in the cytokine array characterization in Amniotic membrane, as analyzed by RayBiotech (average pg/ml).

Each component of birth tissue has a specific function in the development of a fetus. Those tissue components produce cytokines specific to the gestational function they serve. AmnioBind™ includes the intermediate layer which adds even more cytokine and glycoprotein (hyaluronic acid) content. Through our proprietary processing methods, AmnioBind™ proved to have little to no degradation of proteins during processing. The figure below shows the concentration of select proteins measured post processing



PRODUCT PROCESSING

Birth tissue is shipped to Predictive Biotech's lab within 72 hours of birth. Upon arrival, Predictive Biotech's quality assurance team performs inspections to verify that tissue viability has been properly maintained during transport. All tissue is processed in Predictive Biotech's ISO 7 cleanroom under ISO 5 biological safety cabinets. Predictive Biotech's medical director reviews donor's medical records and blood test and deems the donor eligible or ineligible to donate tissue.

PRODUCT SAFETY

AmnioBind™ is manufactured under 21 CFR Part 1271, section 361 of the PHS Act, Current Good Manufacturing Practice (cGMP), Current Good Tissue Practice (cGTP), and is ISO: 13485 accredited. AmnioBind™ is derived from amniotic membranes obtained from donors after normal, full-term pregnancies. Each donor is carefully screened, with comprehensive medical and social histories of donors collected for review by a medical doctor. All tissue is procured, processed and tested in accordance with FDA requirements to minimize potential risks of disease transmission to recipients. Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

DONOR ELIGIBILITY SCREEN		
Test Description	Standards	Status
HIV-1/HIV-2 Antibody	Non-Reactive	Final
HIV-1/HIV-2 NAT	Non-Reactive	Final
Hepatitis B Surface and Core Antibody	Non-Reactive	Final
Hepatitis B NAT	Non-Reactive	Final
Hepatitis C Antibody	Non-Reactive	Final
Hepatitis C NAT	Non-Reactive	Final
Syphilis	Non-Reactive	Final
West Nile Virus	Non-Reactive	Final

Method Description

Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

Figure 5: Donor eligibility screening panel.

Should tissue fail any safety test or the donor eligibility requirements, the entire lot of AmnioBind™ will fail quarantine and be discarded. Once third-party analysis is received and approved, Predictive's quality assurance team conducts a final review of the entire process. If review is approved, the individual lot of product will be released from quarantine. AmnioBind™ is terminally sterilized.

CONCLUSION

AmnioBind™ is processed at Predictive Biotech's state-of-the-art laboratory, where each step is monitored carefully for quality assurance. AmnioBind™ is comprised of cytokines, growth factors, exosomes and scaffolding proteins as validated by third-party labs and internal measures.

The investment in research, processes, equipment, facilities, and third-party testing has provided Predictive Biotech the ability to develop the safest and most consistent placental membrane allografts available in the market. Predictive Biotech's proprietary processing, safety, and normalization standards have positioned AmnioBind™ to be a market leading product in the wound care space.

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