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A Predictive Technology Group Company

WHARTON'S JELLY BIRTH TISSUE 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 in a clinical setting has increased exponentially as clinicians, scientists, researchers and manufacturers continue to innovate and utilize this tissue with patients for its regenerative 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 Wharton's jelly human cell and tissue product (HCT/P), PolyCyte™, is derived from donated, full-term birth tissue. Equipped with state-of-the-art analytical equipment to ensure the safety and viability of its allografts, Predictive's laboratory and proprietary processes maintain the tissue's biological components, yielding a normalized dose. PolyCyte is comprised of cytokines, growth factors, exosomes and scaffolding proteins.

BACKGROUND

Wharton's Jelly

The umbilical cord is a structure that connects the placenta to the developing fetus, serving as a nutrient conduit. At term, in humans, the umbilical cord is typically 40–60 cm long, with a diameter of 1–2 cm. The structure is made of a single layer of amniotic epithelium that encloses a mucoid connective tissue through which three vessels – a vein and two arteries – carry oxygenated and deoxygenated blood between the placenta and fetus, respectively. The three vessels are supported within a layer of connective tissue known as Wharton's jelly.

Function of Wharton's Jelly

Wharton's jelly is classified as connective tissue. It is a gelatinous substance within the umbilical cord largely made up of mucopolysaccharides (hyaluronic acid and chondroitin sulfate). As a mucous tissue, the Wharton's jelly protects and insulates umbilical blood vessels. Extracellular matrix (ECM) components of Wharton's jelly are similar to those of articular cartilage.[1] The ECM is primarily comprised of collagen, which organizes over 50% of the defatted dry weight of tissue. Wharton's jelly also has large amounts of glycosaminoglycans, especially hyaluronic acid, forming about 70% of total glycosaminoglycan content.[2] The hyaluronic acid component found in Wharton's jelly is reported to be higher in molecular weight, 1100± 200 Kda.[3] The high percentage of hyaluronic acid characterizes Wharton's jelly as a strongly hydrated and viscous tissue, suitable as a natural hydrogel-type biomaterial for providing cushioning and lubrication.[4]

PolyCyte Product Description

PolyCyte is a minimally manipulated human tissue allograft derived from Wharton's jelly. PolyCyte is processed to preserve the structural integrity of Wharton's jelly and meets the definition of minimally manipulated as described in the Food and Drug Administration's (FDA) published "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use", for structural tissue (2017). The processing of PolyCyte does not alter the original tissue's function as a Wharton's jelly connective tissue, providing physical support and cushioning. PolyCyte's composition has been analyzed using an in-depth human cytokine array and an enzyme-linked immunosorbent assay (ELISA) array.

CYTOKINES

Cytokines are a broad group of small cellular proteins involved in biological function. 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 products were analyzed by RayBiotech in a Quantibody® analysis, an array-based multiplex ELISA system for simultaneous quantitative measurement of various cytokines, growth factors, proteases, soluble receptors and other proteins. (Figure 1)

Test	Description	PolyCyte avg. pg/mL
CYT5	Ferritin	601,435.3
CYT7	Fetuin A	53,052.9
CYT8	DPPIV	151,431.1
CYT5	Adiponectin	294,627.3
CYT6	APRIL	9,745.0
CYT9	Angiotensinogen	199.0
INF3	TIMP-2	6,690.2
CYT7	TSP-1	85,932.4
CYT9	Periostin	81,723.8
CYT10	CD84	1,008.4
CYT5	ANGPTL4	63,272.2
CYT4	VEGF R1	24,610.1
CYT4	PAI-1	25,018.1
CYT9	Follistatin-like 1	237.1
CYT5	MMP-2	12,260.6
CYT7	VE-Cadherin	1,497.9
CYT9	Thrombospondin-5	14.4
CYT7	Albumin	28,968.9
CYT5	hCGb	2,552.4
CYT7	CD163	31,969.4
CYT9	L1CAM-2	3,057.2
CYT9	DNAM-1	2,210.3
CYT9	LRP-6	3,882.6
CYT10	Pref-1	24,729.2
CYT9	ADAMTS13	1,925.1
CYT7	Furin	2,156.7
INF3	ICAM-1	4,850.4
CYT5	Adipsin	21,394.3
CYT9	CD6	0.0
CYT4	DAN	4,454.5
CYT10	CD155	448.1
CYT7	Syndecan-1	265.4
INF3	TIMP-1	3,607.9
CYT7	RBP4	14,664.0
CYT6	Chemerin	48,162.3
CYT5	Nidogen-1	2,252.9
CYT7	CA19-9	132,302.2
CYT5	NSE	13,810.8
CYT4	Angiostatin	0.0
CYT9	LRIG3	1,603.9
CYT6	CD97	58,961.1
CYT8	bIG-H3	16,358.3
CYT8	IL-10 Ra	0.0
CYT5	Prolactin	13,232.8
CYT10	Siglec-10	3,566.6
CYT9	BMPR-IA	0.0
CYT9	Fractalkine	141.6
CYT6	Transferrin	6,955.5
CYT6	Serpin A4	13,310.0
CYT10	Desmoglein 2	35.0

Test	Description	PolyCyte avg pg/mL
CYT6	Galectin-3	2.4
CYT4	Resistin	696.9
CYT8	IL-5 Ra	2,598.0
CYT5	IGF-1R	2,005.4
REC1	PECAM-1	8,555.6
CYT10	Pentraxin 3	389.1
CYT5	MMP-9	2,457.7
CYT9	FLRG	26,625.0
CYT10	ADAM8	885.2
CYT4	Galectin-7	79.2
CYT10	CD48	1,855.0
CYT9	Persephin	383.9
CYT9	Tie-1	29.5
CYT9	B7-H1	0.0
CYT5	CA125	22,723.0
REC1	ALCAM	1,157.4
CYT6	Thrombomodulin	3,864.3
CYT7	Dkk-3	6,291.7
CYT7	Decorin	6,590.5
CYT8	Cathepsin B	4,440.8
CYT4	DKK-1	233.5
REC1	Trappin-2	4,065.0
CYT6	sFRP-3	1,210.6
CYT7	TRANCE	486.7
REC1	CD14	1,244.9
CYT4	gp130	1,802.0
CYT9	Granulysin	1,385.7
CYT6	Cathepsin L	312.4
CYT10	CEACAM-5	2,710.1
CYT10	SP-D	274.0
CYT5	MMP-1	1,000.3
CYT4	Fcg RIIBC	2,772.1
CYT10	ICOS	943.1
CYT10	CD58	997.0
CYT9	CNTF	942.9
CYT7	ACE-2	503.9
CYT5	CRP	1,628.0
CYT5	Procalcitonin	1,137.7
CYT8	Leptin R	30,908.4
CYT7	AMICA	885.3
CYT10	B7-H3	3,977.0
CYT9	Nectin-4	29.3
CYT10	Cadherin-4	922.8
CYT7	DLL1	3,967.6
CYT8	IL-1 F7	1,361.7
CYT6	CD200	3,090.9
CYT7	IL-17B R	327.7
CYT10	Cystatin B	5,830.4
CYT4	IL-13 R1	227.7
CYT7	CXCL14	1,100.3

Test	Description	PolyCyte avg pg/mL
CYT8	IGFBP-5	2,093.0
CYT4	Siglec-5	566.2
CYT10	Galectin-9	8,398.4
INF3	IL-1ra	248.7
INF3	TNF RI	1,189.1
CYT9	EMMPRIN	5,122.0
CYT6	FAP	8,037.7
CYT6	Neprilysin	7,218.8
CYT6	DcR3	754.0
CYT6	WIF-1	422.6
CYT8	MMP-7	1,087.8
CYT7	WISP-1	429.1
CYT4	E-Cadherin	316.5
CYT6	IGF-2R	5,473.3
REC1	MICB	263.0
CYT4	IL-13 R2	211.2
CYT4	ICAM-2	760.9
CYT8	CHI3L1	8,756.3
CYT7	TACI	195.6
CYT9	IL-17E	183.4
CYT8	PSMA	735.6
CYT10	Galectin-2	1,085.6
CYT7	Clusterin	1,163.2
CYT7	ANG-4	66.1
CYT10	DR3	425.7
CYT5	NCAM-1	2,902.1
REC1	uPAR	315.0
REC1	RAGE	494.8
CYT5	OSM	238.8
REC1	Lipocalin-2	1,757.7
CYT7	RANK	40.1
CYT5	AFP	4,232.1
CYT9	Aggrecan	10.4
CYT7	LAG-3	702.6
CYT5	CA15-3	837.0
CYT5	MMP-13	1,637.2
REC1	VCAM-1	3,498.5
CYT10	ULBP-1	734.5
CYT9	BMPR-II	0.0
CYT5	Thyroglobulin	1,881.6
CYT6	IFNab R2	869.6
CYT6	Legumain	550.3
CYT8	IL-1 F10	61.7
CYT4	FAS L	111.9
CYT8	SIGIRR	656.1
CYT6	HGF R	904.4
REC1	LYVE-1	873.2
CYT9	ULBP-2	101.4
CYT6	C5a	6,091.4
CYT6	IL-1 R6	188.7

Figure 1: Representative list of 150 of the 308 present in the cytokine array characterization in PolyCyte, 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. (Figure 2)

Description	AmnioCyte (avg. pg/mL)	AmnioCyte Plus (avg. pg/mL)	PolyCyte (avg. pg/mL)	CoreCyte (avg. pg/mL)
Fetuin A	138,263	273,162	53,053	345,099
IGFBP-3	67,986	35,281	1,146	2,436
hCGb	55,037	12,458	2,552	0
TSP-1	36,437	55,739	85,932	3,514
IGFBP-6	35,915	17,096	895	1,770
CA15-3	30,540	1,002	837	1,303
SP-D	30,400	2,610	274	49
PAI-1	29,445	24,160	25,018	2,065
DPPIV	24,894	65,702	151,431	19,661
CA125	23,974	3,745	22,723	3,657
APRIL	23,065	61,546	9,745	12,353
Pref-1	20,068	10,552	24,729	484
IGFBP-4	18,401	10,555	2,450	7
FLRG	17,176	4,412	26,625	148
Angiotensinogen	15,421	59,503	199	74,688
Adiponectin	14,970	63,752	294,627	4,766
OPN	14,954	11,487	5	328
VEGF R1	14,530	26,987	24,610	1,012
IGFBP-1	13,371	14,114	5,572	62
CA19-9	11,737	7,677	132,302	617
Thrombospondin-5	11,668	13,237	14	140
Albumin	10,816	12,679	28,969	10,807
TNF RII	10,169	379	825	1,168
Adipsin	9,974	8,744	21,394	2,053
MIF	9,407	28,702	34,833	4,915
MMP-7	8,862	1,383	1,088	0
uPAR	8,527	647	315	69
ICAM-1	8,318	3,892	4,850	6,413
Follistatin-like 1	8,121	22,311	237	9,548
TIMP-2	8,045	6,724	6,690	951
CD97	3,305	7,206	58,961	651
Chemerin	1,858	7,962	48,162	497
Leptin R	0	2,298	30,908	12
Thrombospondin-2	621	673	16,477	150
bIG-H3	7,340	7,075	16,358	3,534
Activin A	68	268	16,245	145
RBP4	7,058	8,132	14,664	8,259
NSE	391	7,658	13,811	4,726
Serpin A4	7,762	5,534	13,310	6,573
Prolactin	3,670	6,345	13,233	244
MCSF R	5,388	2,772	10,079	4,121
Ferritin	265	328,296	601,435	232,582
Periostin	5,239	39,158	81,724	784
CD84	0	38,858	1,008	2,995
ANGPTL4	1,966	36,878	63,272	21,414
MMP-2	4,924	14,746	12,261	3,551
VE-Cadherin	165	14,646	1,498	1,652
PF4	2,202	14,370	38,007	955
CD163	3,238	11,841	31,969	89
L1CAM-2	0	10,970	3,057	61,822
DNAM-1	0	10,868	2,210	258
LRP-6	0	10,646	3,883	483

Figure 2: Quantitative analysis of 52 cytokines present in varying concentrations across Predictive's products. The 52 cytokines are a combination of the 30 highest concentrations for each product

EXOSOMES

Predictive quantified the exosome presence of its products as a part of the product's tissue profile using a standard ELISA (Figure 3). Post-thaw analysis confirmed that PolyCyte contains naturally occurring exosomes from birth tissue and quantified the value to be approximately 4.5 billion exosomes per 1 ml of product. Exosomes are membrane-bound extracellular vesicles that are present in all bodily fluids under both normal and pathophysiological conditions8. Exosomes are produced in the endosomal compartment of most eukaryotic cells. An ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.

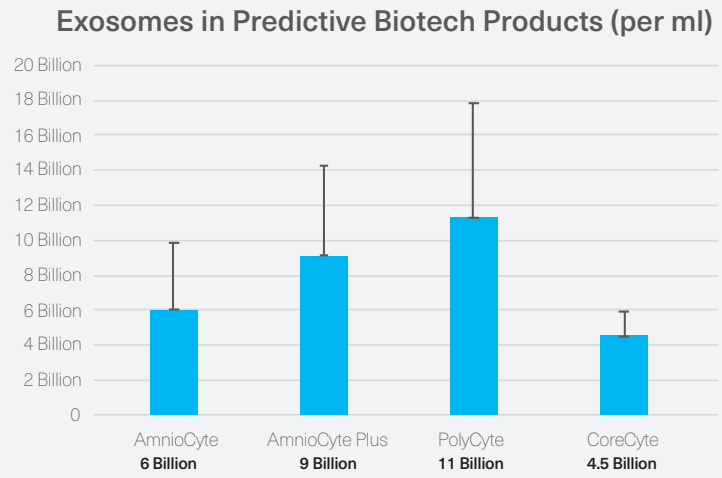

Figure 3: Quantitative analysis of exosomes in Predictive products was performed using an ExoQuick-TC Ultra kit to isolate the exosomes and then was quantified using the ExoELISA-ULTRA Complete Kit. Quantification was accomplished using a spectrophotometric plate reader (Thermo MultiSkan FC) at 450 nm.

PRODUCT SAFETY

PolyCyte is manufactured under Current Good Manufacturing Practice (cGMP), Current Good Tissue Practice (cGTP) and is ISO 13485 accredited. PolyCyte is derived from Wharton's jelly 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.

PRODUCT SAFETY PROCESS

Birth tissue is shipped to Predictive's lab within 72 hours of birth. Upon arrival, Predictive's quality assurance team performs inspections to verify that tissue viability has been properly maintained during transport. All tissue is processed in Predictive's ISO 7 cleanroom under ISO 5 biological safety cabinets. Once produced, three (3) samples of each lot of PolyCyte are sent for safety testing. All remaining product is placed into cryogenic quarantine (release pending third-party validation and medical director approval). Two (2) of the samples are sent to a third-party laboratory for serology and bacterial endotoxin testing. The third sample is screened in-house for particulates using a Horizon Halo system for subvisible particle analysis in order to meet USP 790 standards. (Figure 4)

14 Day Microbial Screening

Sample ID	Product Description	Result	Status
S-012820-05826	C20023007	No Growth, Day 14	Final

Method Description

14-day microbial screening in accordance with USP <71>, 21CFR610.12.

Microbial screening looks for bacterial, fungal and/or mold growth. If growth present in the sample, the entire lot will be discarded and the growth will be characterized to understand the type of contamination.

Bacterial Endotoxin Test

Sample ID	Result Units	Result	Status
S-012820-05826	EU/ml	0.0718	Final

Method Description

Endotoxin testing in accordance with USP <85>, ANSI / AAMI ST72:2011.

Endotoxins are bacterial structural components that are released when such a cell is lysed. These components are toxic if administered to humans and animals, causing a pyrogenic response (rise in body temperature). All samples must have an EU/ml of less than 3.0.

Particulate Test

Halo System - Background membrane imaging

Clean Brightfield | Failed Brightfield | Clean Simi View | Failed Simi View

Method Description

Particulate testing in accordance with USP <787>, USP <788>, USP <790>.

Figure 4: Safety tests (microbial screening, bacterial endotoxin, and particulate) are performed on every lot of CoreCyte. Sample results.

Predictive's medical director reviews donor's medical records and blood test, and deems the donor eligible or ineligible to donate tissue.

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
Cytomegalovirus IgM	Not Detected	Final
Microbial Screening – Bacterial	No Growth (USP 71)	Final
Microbial Screening – Fungal	No Growth (USP 71)	Final
Bacterial Endotoxin (BET)	≤3 EU/ml	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 (microbial screening or endotoxin) or the donor eligibility requirements, the entire lot of AmnioCyte Plus 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. AmnioCyte Plus is shipped overnight on dry ice to keep product at appropriate temperature to maintain product viability.

CONCLUSION

PolyCyte is processed at Predictive Biotech's state-of-the-art laboratory, where each step is monitored carefully for quality assurance. PolyCyte 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 the ability to develop the safest and most consistent Wharton's jelly derived allografts available in the market. With over 100,000 allografts shipped, Predictive's processing, safety, and normalization standards have established PolyCyte as a market leading product.

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