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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), CoreCyte™, 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. CoreCyte is primarily comprised of pericytes, mesenchymal stem cells (MSCs), cytokines, growth factors, and exosomes.

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 glycosaminoglycans 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]

CoreCyte Product Description

CoreCyte is a minimally manipulated human tissue allograft derived from Wharton's jelly. CoreCyte 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 CoreCyte does not alter the original tissue's function as a Wharton's jelly connective tissue, providing physical support and cushioning.

CoreCyte has undergone 11 process changes (since its launch) in pursuit of reducing variation and increasing the quality and safety of each allograft, while maintaining the tissue's biological components. CoreCyte's composition has been analyzed using flow cytometry, an in-depth human cytokine array, an enzyme-linked immunosorbent assay (ELISA) array, and an extracellular matrix characterization.

For regulatory purposes, CoreCyte is:

- minimally manipulated
- not combined with other articles, exception of a cryogenic preservative (DMSO)
- not dependent on metabolic activity of living cells for product's primary function
- designated as an HCT/P under CFR 1271 Section 361

CELLULAR CHARACTERIZATION

Cells are classified by the type of work they do within the environment in which they are found. Visually, cells can be classified either by 1) the cell's shape, or 2) surface markers, or uniforms, which are identical across cells that serve the same function. However, in most situations, it is difficult to classify cells by normal visual means due to the microscopic size of cells and the vast number of cell subtypes.

Predictive Biotech aliquots approximately 2.5 million nucleated cells in each 1 ml unit of CoreCyte (after manufacturing). Flow cytometry allows for the rapid identification, separation and characterization of various cell subtypes by virtue of size and morphology from a fluid heterogeneous mixture. This process uses antibodies to fluorescently label individual cells in a sample.

CORECYTE STANDARDIZATION EXAMPLE

Umbilical Cord	Cord Length	Flow Cytometry Evaluation	Aliquot (2.5M)
Cord 1	9 inches	120 million viable cells	48 - 1 ml vials
Cord 2	12 inches	20 million viable cells	8 - 1 ml vials
Cord 3	10 inches	75 million viable cells	30 - 1 ml vials

Figure 1: Flow cytometry analysis is used during processing to determine the yield of each individual cord. The cord is then aliquoted to this measure, reducing unit variation between cords.

Flow cytometry is used to analyze each umbilical cord to determine cell count and cell viability. With this data, Predictive normalizes CoreCyte to provide approximately 2.5 million nucleated cells per 1 ml of product. This product is cryopreserved at -180 degrees Celsius to maintain cell viability.

CoreCyte is aliquoted to contain approximately 1 million (+/- 150k) viable MSCs per 1 ml of allograft material after being thawed (from cryopreservation) and ready for administration by provider. The native tissue MSCs identified in CoreCyte are characterized using positive markers CD29, CD73 and CD105, and negative markers CD14, CD34, CD45, CD19, CD11b, and HLA-DR. Although the International Society for Cell & Gene Therapy (ISCT) standards also identify CD90 as a positive marker for MSCs, these standards apply to cultured cells, and as such do not accurately represent native MSCs found in birth tissue.[5] From scientific literature and experiments conducted, Predictive agrees that CD29 is a positive marker for native tissue MSC characterization in a heterogeneous mix. CD29 is also accepted by the FDA for investigational new drug (IND) product characterization submissions.[6,7]

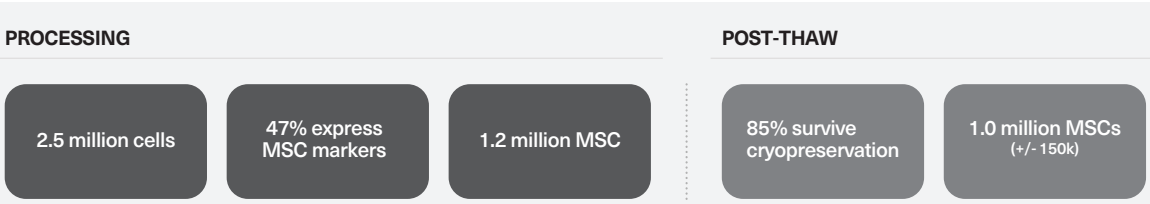

Figure 2: Predictive's standard operating procedure directs flow cytometry be used on each processed umbilical cord to standardize units of product to 1.0 million MSCs per 1 ml. CoreCyte MSCs are characterized using markers: CD29+, CD105+, CD73+, CD14-, CD34-, CD45-, CD19-, CD11b-, HLA-DR-.

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 3**).

Test	Description	CoreCyte (avg. pg/ml)
CYT5	Ferritin	232,582.1
CYT7	Fetuin A	345,099.0
CYT8	DPPIV	19,660.7
CYT5	Adiponectin	4,766.2
CYT6	APRIL	12,353.0
CYT9	Angiotensinogen	74,688.4
INF3	TIMP-2	950.8
CYT7	TSP-1	3,513.6
CYT9	Periostin	784.4
CYT10	CD84	2,994.8
CYT5	ANGPTL4	21,414.0
CYT4	VEGF R1	1,011.8
CYT4	PAI-1	2,065.2
CYT9	Follistatin-like 1	9,548.3
CYT5	MMP-2	3,550.7
CYT7	VE-Cadherin	1,651.6
CYT9	Thrombospondin-5	140.5
CYT7	Albumin	10,807.1
CYT5	hCGb	0.0
CYT7	CD163	89.4
CYT9	L1CAM-2	61,821.7
CYT9	DNAM-1	258.0
CYT9	LRP-6	483.3
CYT10	Pref-1	484.2
CYT9	ADAMTS13	52.8
CYT7	Furin	106.3
INF3	ICAM-1	6,413.0
CYT5	Adipsin	2,053.0
CYT9	CD6	17.8
CYT4	DAN	251.4
CYT10	CD155	10,817.5
CYT7	Syndecan-1	890.7
INF3	TIMP-1	2,216.4
CYT7	RBP4	8,258.7
CYT6	Chemerin	497.3
CYT5	Nidogen-1	265.3
CYT7	CA19-9	616.6
CYT5	NSE	4,726.1
CYT4	Angiostatin	557.5
CYT9	LRIG3	74.0
CYT6	CD97	650.7
CYT8	bIG-H3	3,533.7
CYT8	IL-10 Ra	300.4
CYT5	Prolactin	243.7
CYT10	Siglec-10	768.4
CYT9	BMPR-IA	155.5
CYT9	Fractalkine	0.0
CYT6	Transferrin	1,265.5
CYT6	Serpin A4	6,573.2
CYT10	Desmoglein 2	4.7
CYT6	Galectin-3	410.8
CYT4	Resistin	121.2
CYT8	IL-5 Ra	0.0
CYT5	IGF-1R	95.8
REC1	PECAM-1	90.6
CYT10	Pentraxin 3	10.8
CYT5	MMP-9	125.5
CYT9	FLRG	147.7
CYT10	ADAM8	40.8
CYT4	Galectin-7	1,587.4
CYT10	CD48	868.2
CYT9	Persephin	72.9
CYT9	Tie-1	38.9
CYT9	B7-H1	49.0
CYT5	CA125	3,657.0
REC1	ALCAM	461.5
CYT6	Thrombomodulin	1,261.9
CYT7	Dkk-3	94.9
CYT7	Decorin	2,016.1
CYT8	Cathepsin B	39.1
CYT4	DKK-1	51.5
REC1	Trappin-2	213.2
CYT6	sFRP-3	91.2
CYT7	TRANCE	0.0
REC1	CD14	75.0
CYT4	gp130	150.9
CYT9	Granulysin	30.2
CYT6	Cathepsin L	121.5
CYT10	CEACAM-5	1,192.1
CYT10	SP-D	48.7
CYT5	MMP-1	44.9
CYT4	Fcg RIIBC	342.6
CYT10	ICOS	148.9
CYT10	CD58	143.6
CYT9	CNTF	309.4
CYT7	ACE-2	0.0
CYT5	CRP	22.7
CYT5	Procalcitonin	0.0
CYT8	Leptin R	11.5
CYT7	AMICA	2.8
CYT10	B7-H3	357.7
CYT9	Nectin-4	79.7
CYT10	Cadherin-4	173.5
CYT7	DLL1	159.5
CYT8	IL-1 F7	357.4
CYT6	CD200	306.2
CYT7	IL-17B R	0.0
CYT10	Cystatin B	1,164.4
CYT4	IL-13 R1	636.7
CYT7	CXCL14	40.7
CYT8	IGFBP-5	89.8
CYT4	Siglec-5	283.3
CYT10	Galectin-9	354.1
INF3	IL-1ra	13.3
INF3	TNF RI	1,860.8
CYT9	EMMPRIN	363.2
CYT6	FAP	587.7
CYT6	Neprilysin	811.7
CYT6	DcR3	135.7
CYT6	WIF-1	58.4
CYT8	MMP-7	0.0
CYT7	WISP-1	15.0
CYT4	E-Cadherin	98.6
CYT6	IGF-2R	283.3
REC1	MICB	10.4
CYT4	IL-13 R2	72.0
CYT4	ICAM-2	13,750.8
CYT8	CHI3L1	150.4
CYT7	TACI	0.0
CYT9	IL-17E	18.6
CYT8	PSMA	0.0
CYT10	Galectin-2	57.8
CYT7	Clusterin	600.8
CYT7	ANG-4	128.2
CYT10	DR3	8.1
CYT5	NCAM-1	244.0
REC1	uPAR	69.0
REC1	RAGE	0.0
CYT5	OSM	59.4
REC1	Lipocalin-2	666.3
CYT7	RANK	67.4
CYT5	AFP	605.3
CYT9	Aggrecan	0.8
CYT7	LAG-3	98.9
CYT5	CA15-3	1,303.0
CYT5	MMP-13	792.3
REC1	VCAM-1	644.7
CYT10	ULBP-1	85.0
CYT9	BMPR-II	0.0
CYT5	Thyroglobulin	0.0
CYT6	IFNab R2	49.9
CYT6	Legumain	32.0
CYT8	IL-1 F10	0.0
CYT4	FAS L	16.3
CYT8	SIGIRR	31.4
CYT6	HGF R	6.9
REC1	LYVE-1	433.7
CYT9	ULBP-2	51.6
CYT6	C5a	11.0
CYT6	IL-1 R6	130.6

Figure 3: Representative list of 150 of the 171 present in the cytokine array characterization in CoreCyte, as analyzed by RayBiotech (average pg/ml).

EXOSOMES

Predictive quantified the exosome presence of its products as a part of the product's tissue profile using a standard ELISA (**Figure 4**). Post-thaw analysis confirmed that CoreCyte 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 conditions.[8] 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.

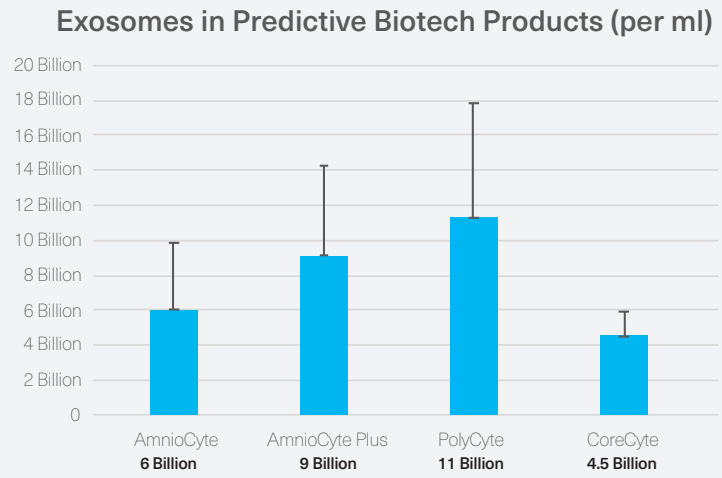

Figure 4: Quantitative analysis of exosomes in Predictive products was performed using an ExoQuick-TC Ultra kit to isolate the exosomes and then was quantified using the ExoELISA-ULTRA Complete Kit. Quantification was accomplished using a spectrophotometric plate reader (Thermo MultiSkan FC) at 450 nm.

PRODUCT SAFETY

CoreCyte is manufactured under Current Good Manufacturing Practice (cGMP), Current Good Tissue Practice (cGTP) and is ISO 13485 accredited. CoreCyte 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 CoreCyte 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.

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 5: 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
Particle Contaminant	USP 787, 788, 790	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 6: Donor eligibility screening panel.

Should tissue fail any safety test (microbial screening, endotoxin, and particulate) or the donor eligibility requirements, the entire lot of CoreCyte 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. CoreCyte is shipped overnight on dry ice to keep product at appropriate temperature to maintain product viability.

CONCLUSION

CoreCyte is processed at Predictive Biotech's state-of-the-art laboratory, where each step is monitored carefully for quality assurance. CoreCyte is comprised of mesenchymal stem cells (MSCs), 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 CoreCyte as a market leading product.

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