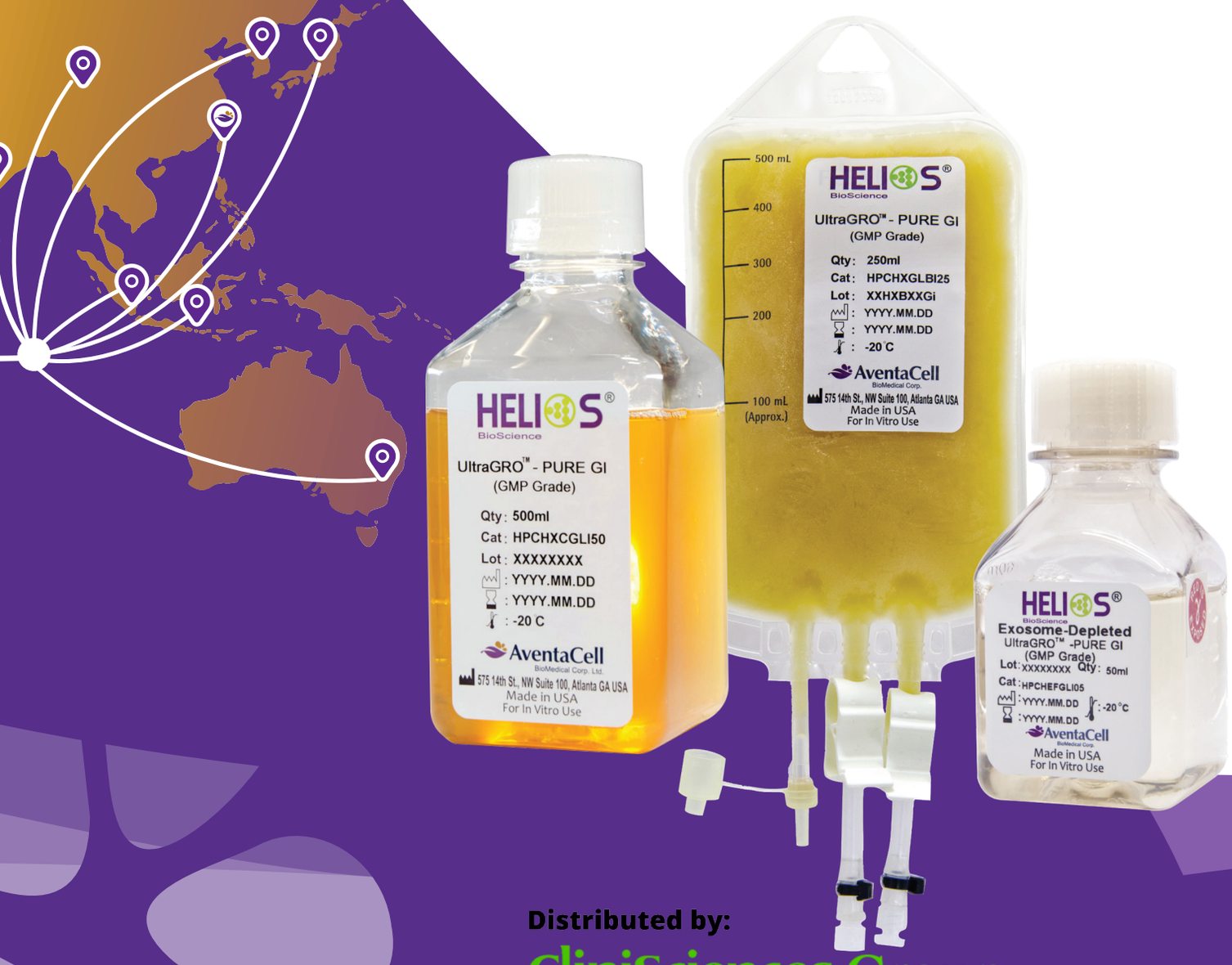
AventaCell
BioMedical Corp

Enhance Your Cell Culture Performance with an UltraGRO™ hPL Supplement



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WELCOME TO AVENTACELL BIOMEDICAL CORP.

AventaCell BioMedical Corp. ("AventaCell") is among the world leaders devoted to developing novel human-derived products for use in cell culture and tissue regeneration. Helios Bioscience is AventaCell's product family brand for their products to be used in cell culture and tissue regeneration. AventaCell technologies used in the Helios Bioscience line offer new human-derived solutions for use in translational research of cell and tissue-based therapies to meet the need for animal serum-free cell expansion and production. The demand for safe, efficient and cost-effective cell expansion and production is rapidly increasing with the growth in cell therapy and regenerative medicine research and clinical development. Helios Bioscience products are designed to support expansion and production of a broad range of cells including mesenchymal stem cells and multiple immunocell lines. AventaCell is committed to providing animal serum-free products to accelerate the research, development and commercialization of safe and efficacious cell and tissue-based therapeutics.

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UltraGRO™ Product Lines Overview:

Exosome-Depleted Human Platelet Lysate (xeno-free)

NEW

Exosome-Depleted UltraGRO™-PURE GI

Fibrinogen-depleted Human Platetlet Lysate (xeno-free)

UltraGROTM-PURE GI

US FDA DMF #33284New
Japan PMDA Certificate
Ph. Eur. General Chapter 5.2.12.2.4 Compliance

UltraGRO™-PURE

US FDA DMF #33983

Fibrinogen-depleted Human Platelet Lysate (non-xenogeneic)

UltraGRO™-Advanced GI

Japan PMDA Certificate
Ph. Eur. General Chapter 5.2.12.2.4 Compaliance

UltraGRO™-Advanced

US FDA DMF # 33614

Fibrinogen-rich Human Platelet Lysate (xeno-free)

UltraGRO™

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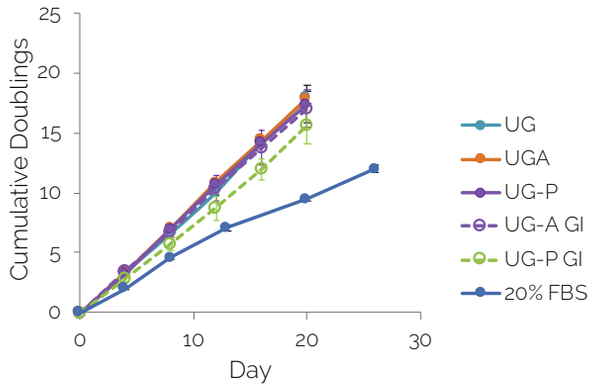
Cell Culture with UltraGRO™ Product Lines

Human platelet lysate (hPL) has been identified in numerous studies to be an effective, preferred xeno-free cell culture supplement to replace FBS. It contains abundant growth factors and cytokines necessary for cell proliferation and is increasingly used globally as an ancillary material in clinical protocols for cell and gene therapy (CGT) product production. GMP-compliant hPL products are manufactured from pooled human platelets collected from healthy donors at FDA-licensed blood centers, and each donor is tested in compliance with FDA guidance. Potency is highly consistent when pooling hundreds of platelet units for each lot, achieving minimal batch-to-batch variation.

BENEFITS OF ULTRAGRO™

- Non-xenogeneic/ xeno-free serum substitute
- No adhesion factor needed
- Replace 10-20% Fetal Bovine Serum (FBS) with 5% UltraGRO product lines
- Better performance than FBS in primary isolation and expansion cultures
- Shorten population doubling times (20-30 hrs)
- Research and GMP grade for seamless bench to bedside transition
- Large batch production to minimize batch-to-batch variability

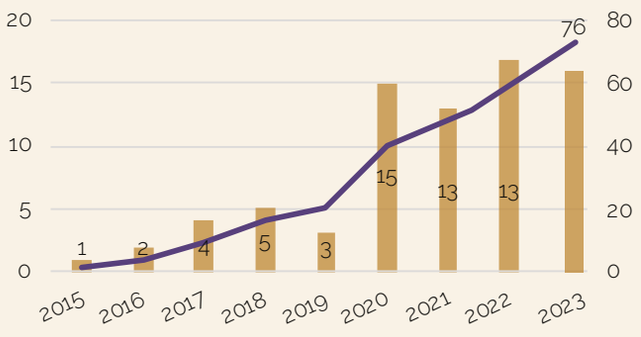
5% UltraGRO™ product lines in ADMSC cultures

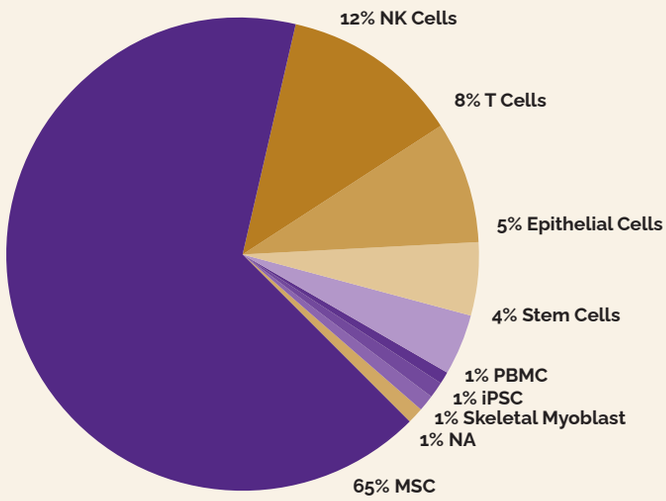


UltraGRO™ Product Lines in Patents

76 Registered Patents

Patents registered with UltraGRO™





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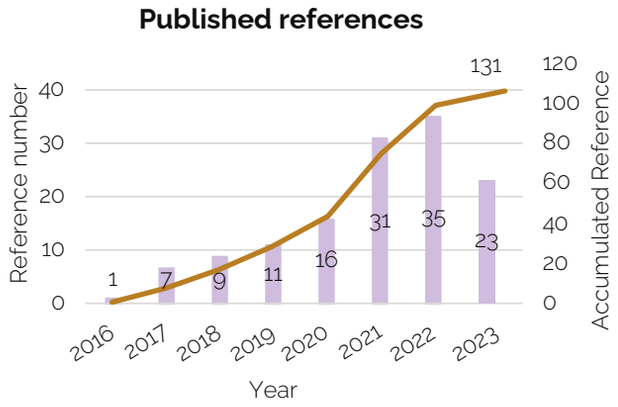
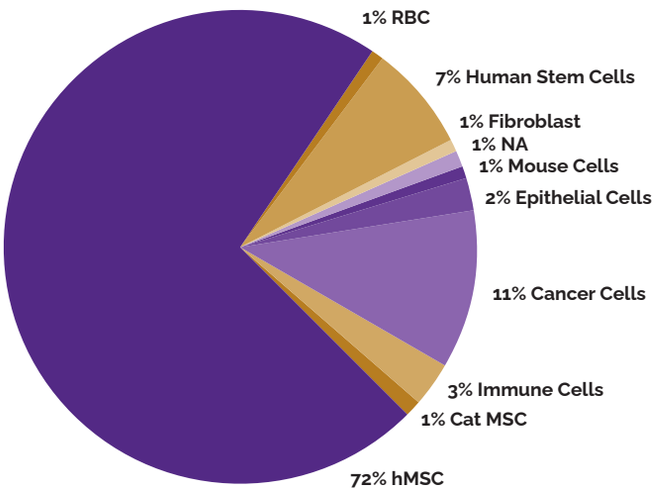
Published UltraGRO™ Product Lines Applications

131 References / A Total of 32 Cell Lines

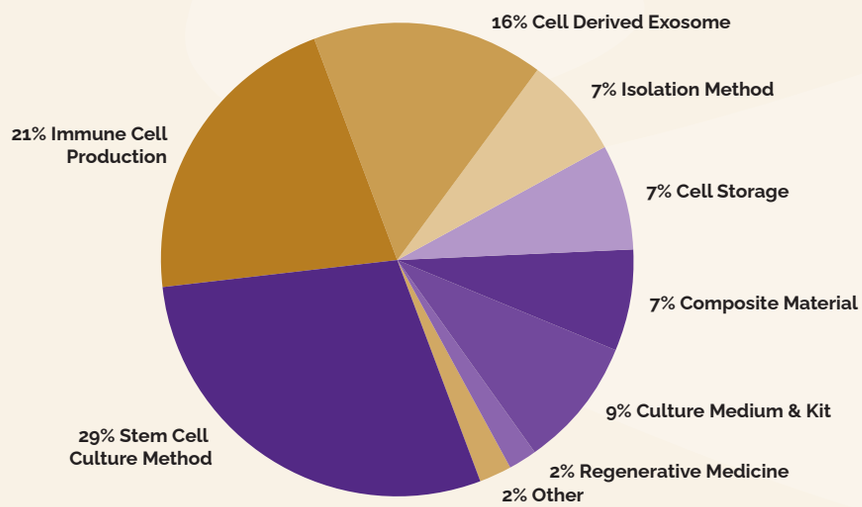
Use with Confidence!

Academic publications from all around the world are using UltraGRO™ product lines for biomedical research!





Cell Line Applications			
1	UCMSC	17	Primary T
2	ADMSC	18	PBMC
3	BMMSC	19	A2780
4	Amniotic-derived MSC	20	HEY
5	Chorionic villous-derived MSC	21	HO8910
6	WJ-MSC	22	GBM
7	Placenta-derived MSC	23	A549
8	ADSC	24	HUVECs
9	Skeletal stem cells	25	Hs68 cells
10	Corneal epithelial cell	26	OVCAR4
11	Primary human foreskin fibroblasts Leydig-like cells	27	mouse Neuro-2a cell line
12	mouse MSC	28	Hela
13	Circulating tumor cells	29	SNK-6 cells
14	PBMC-derived DC	30	SNK-6
15	RBC	31	ADM-SP
16	SKOV3	32	Cat MSC



UltraGRO™ product lines have been applied on different kinds of cells for 9 innovated therapeutic sectors of patent registrations!

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Gamma Irradiation of Human Platelet Lysate: Validation of Efficacy for Pathogen Reduction and Assessment of Impacts on hPL Performance

Gamma irradiation is one of the most widely employed methods for pathogen reduction and commercial gamma sterilization facilities are easily accessible. The whole system for manufacturing gamma irradiated fetal bovine serum (FBS) has been well-established, including dose range, dose mapping, frozen condition, as well as validation of pathogen reduction. Nevertheless, many research articles have addressed the optimal conditions for utilizing gamma irradiation in human plasma and blood components. With these comprehensive references, we previously assessed the feasibility of using gamma irradiation to obtain pathogen-reduced human platelet lysate (hPL) and reported low impacts on the potency for cell expansion.

In this study, we validated the efficacy of gamma irradiation for virus inactivation. Four model viruses (BVDV, Reo3, HSV1, MMV) were chosen, per ICH/EMA guidelines, to represent a range of viruses with different genome, structure, size, and sensitivity to various chemical and physical agents. The virus spiked hPLs were gamma irradiated and the mean values of viral titers showed more than 4 log10 reduction across all model viruses. The results demonstrated gamma irradiation is an effective viral reduction procedure for hPL.

To assess the impacts of gamma irradiation on the long-term stability of hPL performance, we analyzed UltraGRO™ GI series up to one year after gamma irradiation. The results showed growth factors still retained comparable levels to the non-irradiated hPLs. Mesenchymal stromal cells (MSC) cultured with gamma irradiated hPLs for more than three passages showed similar profiles as with the corresponding non-irradiated hPLs in respect of growth rate, morphology, immunophenotype, trilineage differentiation potency, and immunosuppressive property.

COMPARISON OF PATHOGEN REDUCTION TREATMENT (PRT) FETAL BOVINE SERUM (FBS) VS. ULTRAGRO™ GI SERIES VIRAL CLEARANCE VALIDATION

PRT FBS	Vs.	UltraGRO™ GI Series
0.22µm	Sterile filtration	0.22µm
<-10° C	Finished products storage	-20° C
Frozen	Transportation to irradiation plant	Frozen on dry ice
Gamma	Irradiation	Gamma
Cobalt-60	Radiation source	Cobalt-60
5-60 kGy (viral inactivation study) 25-40 kGy (typically employed for commercial products)	Dosage	25-40 kGy
Sealed containers	Physical state	Sealed bottles
Dry ice	Temperature control	Dry ice
Frozen	Transportation to supplier storage	Frozen on dry ice

Virus Category	RNA	RNA	DNA	DNA
	Enveloped	Non-Enveloped	Enveloped	Non-Enveloped
Model for	HCV, HIV	HAV	CMV, EBV, HBV	B19
Virus	BVDV	Reo3	HSV1	MMV
Family	Flavi	Reo	Herpes	Parvo
Genome	ssRNA	dsRNA	dsDNA	ssDNA
Size (nm)	40-60	60-80	120-200	18-24
Resistance	Low	Med-High	Medium	Very High
UltraGRO™-PURE GI	> 5.42	> 4.40	> 4.51	4.55
UltraGRO™ -Advanced GI	> 5.54	> 4.27	> 4.50	4.46

MSCs Culture with UltraGRO™-PURE GI GMP

Gamma Irradiated/ Viral Inactivate
Xeno-free, Safe, Consistent, Cost effective cell culture

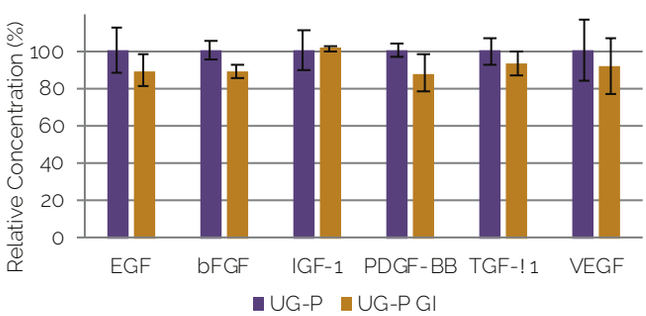
BENEFITS OF ULTRAGRO™ PURE GI

- **US FDA DMF # 34284**
- **JAPAN PMDA Certificate**
- **Ph. Eur. General Chapter 5.2.12.4 Compliance**
- UltraGRO™-PURE GI supplements for producing clinical grade cells
- Gamma irradiation has been accepted by regulatory agencies as a validated PRT
- Comparable cell culture performance maintained
- Viral inactivated products without loss of potency

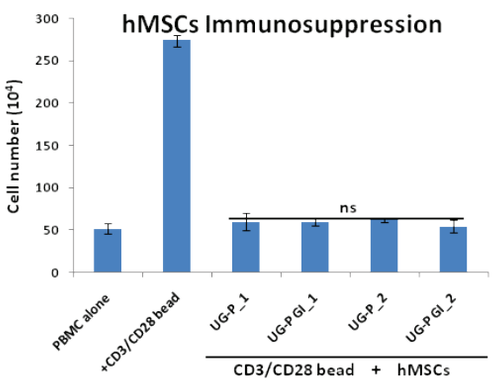
AventaCell BioMedical has adopted a state-of-the-art gamma irradiation process, as a pathogen reduction treatment (PRT), for viral inactivation to create an UltraGRO™-PURE GI (UG-P GI) product. UG-P GI offers minimized pathogen contamination risk while preserving potent cell culture performance with human mesenchymal stem cells (hMSCs), human immune cells and other applicable cell types for clinical applications.

Marker %	Cell type	CD73	CD90	CD105	CD34	CD45	CD11b	CD79a	HLA-DR
UG-P GI	AD-MSC	99.97	99.88	95.33	0.34	0.40	0.78	0.37	1.65
	UC-MSC	95.51	99.98	99.09	0.80	0.31	1.08	1.11	1.97
	BM-MSC	99.94	99.50	99.95	0.93	0.15	0.15	0.34	1.45

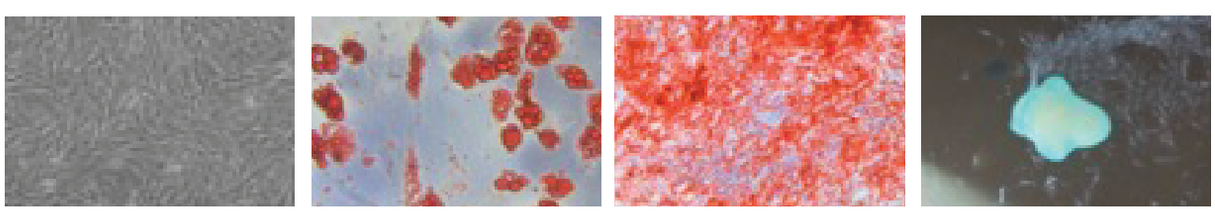
Immuno-phenotypical characterization of human MSCs. Human MSCs derived from adipose tissue (AD), umbilical cord matrix (UC), bone marrow (BM) cultured in UltraGRO™-PURE GI for 5 passages displayed characteristic expression of MSC surface markers.



Growth factors retained comparable cytokine levels after receiving gamma irradiation



MSCs retained immunomodulation potency



AD MSC | Adipo-genesis | Osteo-genesis | Chondro-genesis

Human adipose tissue derived MSCs retain tri-lineage differentiation capability after cultured in UltraGRO™-PURE GI supplemented medium for three passages

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Exosome-Depleted UltraGRO™-PURE GI

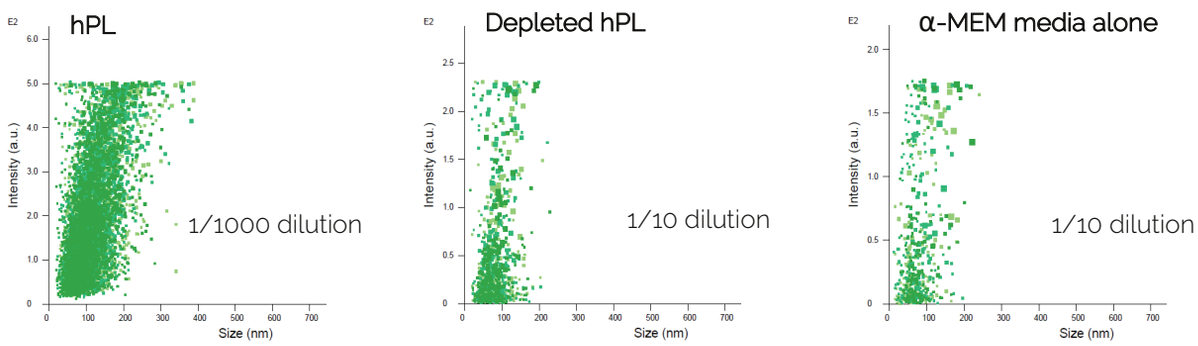
For Therapeutic Cell-derived EV production

NEW

AventaCell BioMedical Corp. has developed an exosome depletion process to remove human platelet lysate (hPL)-derived exosomes. Exosome-Depleted UltraGRO™-PURE GI (ED UG-P GI) is able to support human MSC cell viability to secret abundant extracellular vesicles (EVs) without compromising phenotype over the culture period. Moreover, gamma irradiation processing of the product is used as a pathogen reduction treatment (PRT) for viral inactivation, to comply with regulatory guidance for clinical research and development.

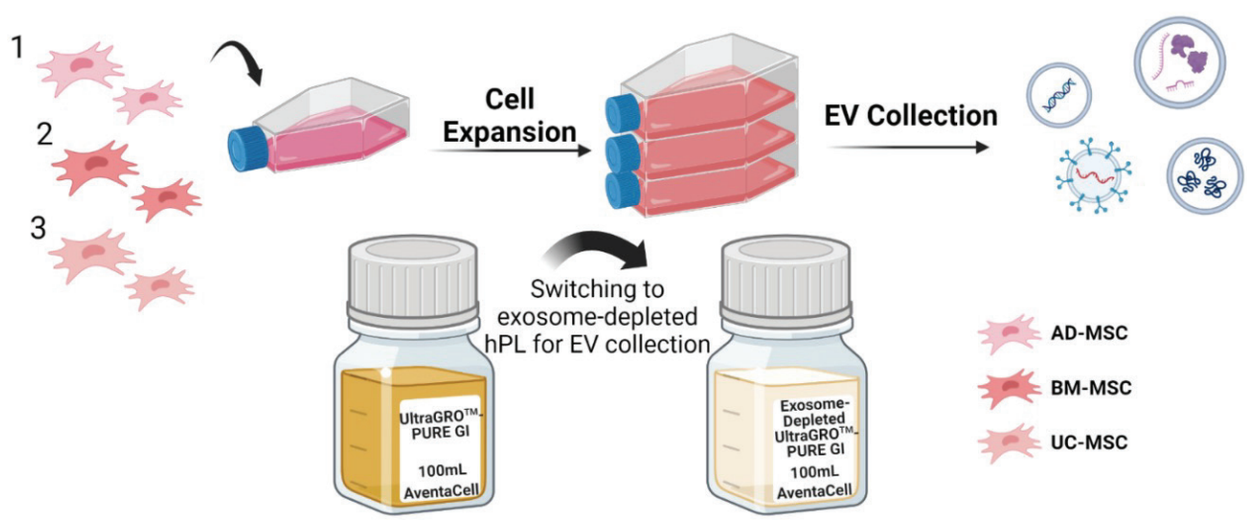
BENEFITS OF EXOSOME-DEPLETED ULTRAGRO™-PURE GI

- Xeno-free with >95% nanoparticle removal from the hPL supplement
- Minimal hPL nanoparticle contamination
- MSCs cultured with the depleted supplement remain highly viable with stable phenotype markers throughout the culture period
- Exosome-Depleted UltraGRO™-PURE GI to produce hMSC-derived EVs
- Gamma irradiation processing is accepted by regulatory agencies as a validate



Nanoparticles were analyzed by NTA. Nanoparticle size distribution in hPL product before and after the depletion process compared to α-MEM basal media alone. Results showed a significant particle removal of the particle signal after the depletion process. Moreover, the outstanding and consistent particle removal from each batch was performed in the study, resulting an average of 99% of depletion rate (n = 5).

NTA results (n = 5)	Non-depleted hPL	Depleted hPL	Depletion rate
Particle count/mL	$2.73 \times 10^{11} \pm 6.48 \times 10^{10}$	$3.52 \times 10^{9} \pm 3.16 \times 10^{9}$	99.07%



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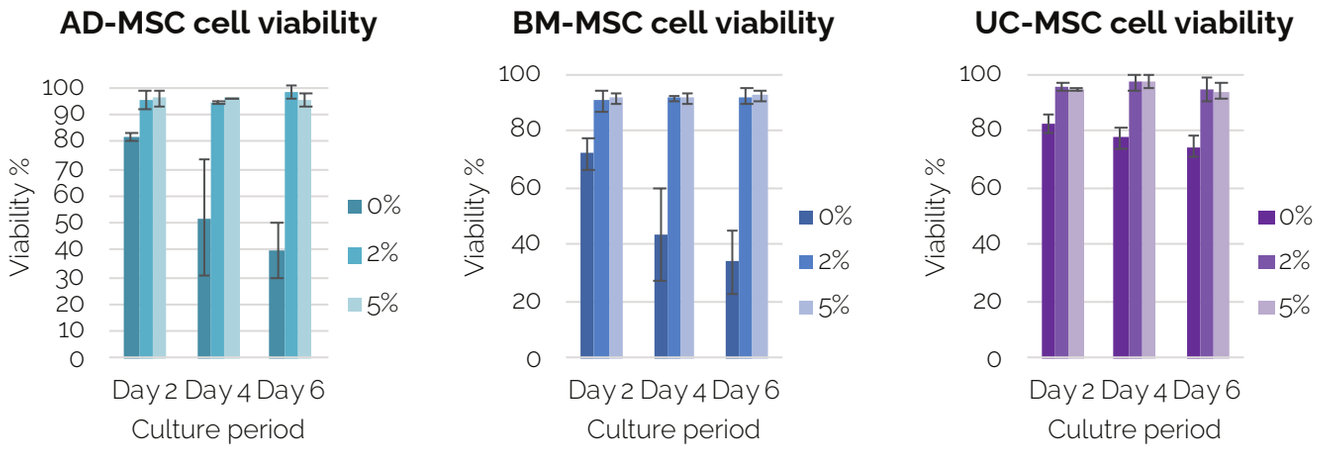
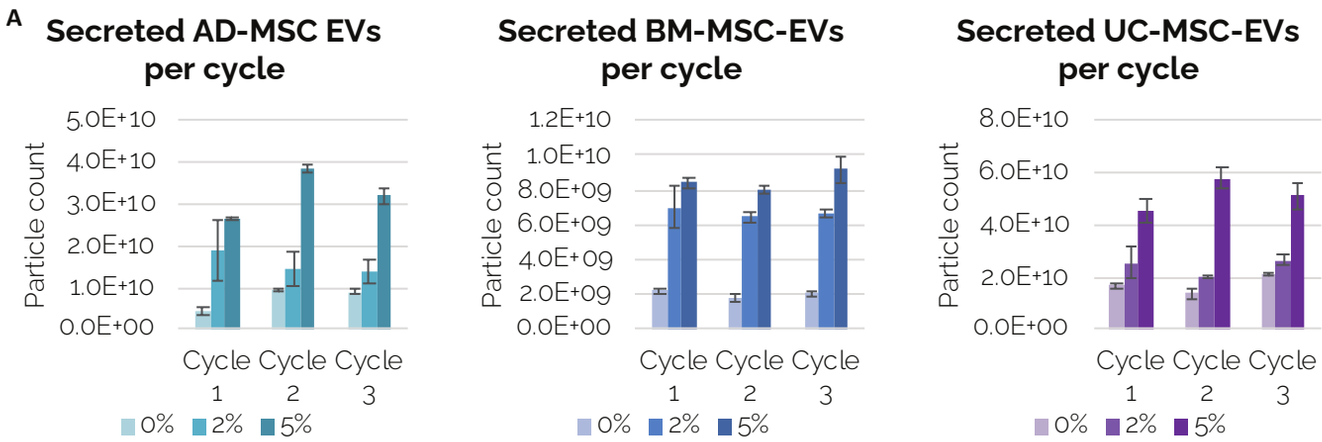
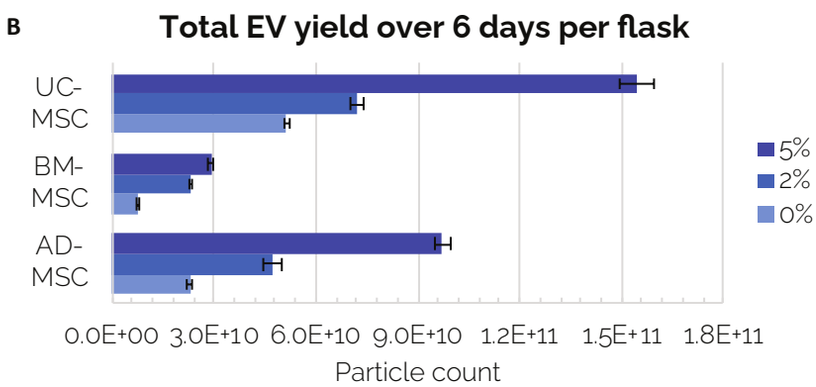
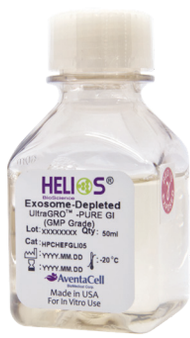


Cell viability of AD-, BM-, and UC-MSCs were monitored during the culture period. Target MSCs cultured in the presence of ED UG-P GI showed >90% viable with continuous growth compared to α-MEM basal alone which presented a significant drop on day 2.







Secreted MSC-derived EVs were collected from each production cycle and were analyzed by NTA. (A) EV secretion profile in each production cycle, (B) total MSC-derived EV yield per T75 flask in 6 days

Product Number	Product	Bottle Size (mL)
HPCHEFRLI05	Exosome-Depleted UltraGRO™-PURE GI	50
HPCHEFRLI10		100
HPCHEFRLI50		500
HPCHEFGLI05	Exosome-Depleted UltraGRO™-PURE GI (GMP grade)	50
HPCHEFGLI10		100
HPCHEFGLI50		500

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UltraGRO™-PURE GI in INF-α-induced Dendritic Cells Generation with High Endocytic and Proteolytic Activities for Immunotherapies

Interferon-α-Induced Dendritic Cells Generated with Human Platelet Lysate Exhibit Elevated Antigen Presenting Ability to Cytotoxic T Lymphocytes

Ippei Date[1],Terutsugu Koya[1,2],Takuya Sakamoto[1,2],Misa Togi[1,2],Haruhiko Kawaguchi[1],Asuka Watanabe[1],Tomohisa Kato, Jr.[3] and Shigetaka Shimodaira[1,2]

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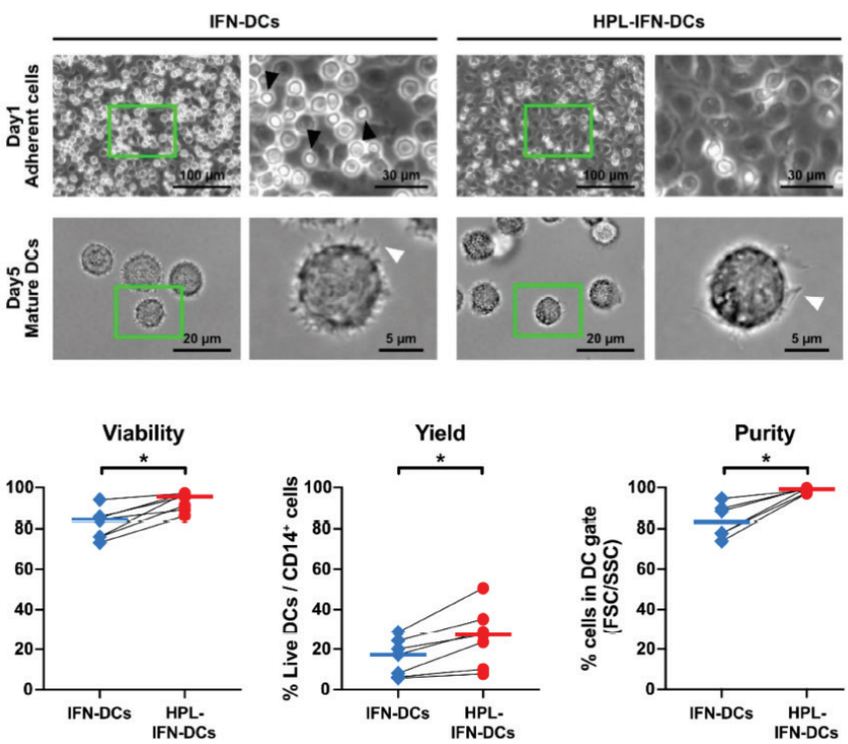
[3] Medical Research Institute, Kanazawa Medical University, Uchinada, Kahoku 920-0293, Japan

The viability, yield, and purity in IFN-DCs and HPL-IFN-DCs. The upper panels display micrographs of cells seeded on adherent dishes (magnification: 10, scale bar: 100 and 30 m). The black arrowheads indicate small cells, such as lymphocytes. The lower panels display micrographs of the harvested mature DCs in a glass bottom dish (magnification: 40, scale bar: 20 and 5 m). The white arrowheads indicate dendrite-like structures. A partial close-up is shown to the right of each photo (green squares). Dead cells were measured by trypan blue staining to compare the viability and yield of the DC/monocyte ratio. DC purity was measured by flow cytometry. The gated cells from FSC and SSC, excluding the lymphocyte fraction, were defined as DCs (viability and yield, n = 7; purity, n = 6). The bold horizontal bars in the graphs indicate the median of each parameter. * $p < 0.05$

Reference: Date, Ippei, et al. "Interferon-α-induced dendritic cells generated with human platelet lysate exhibit elevated antigen presenting ability to cytotoxic T lymphocytes." Vaccines 9.1 (2020): 10.



Viability and yield of IFN-DCs in DCO-K only, with human AB serum, and with HPL conditions.

	DCO-K only	+ human AB serum	+ HPL*
Yield (DC/Monocyte)	14.3%	9.7%	25.2%
Viability	85.5%	61.4%	95.5%

* human platelet lysate.

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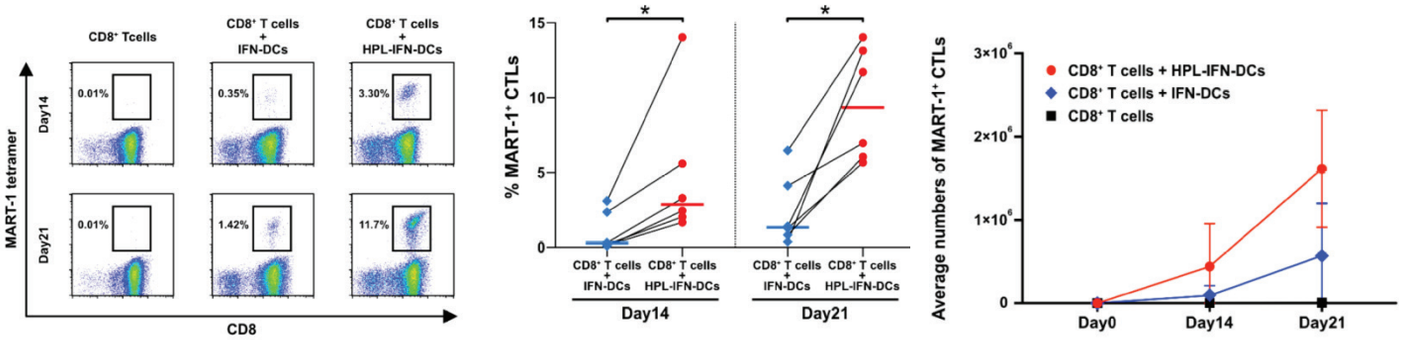
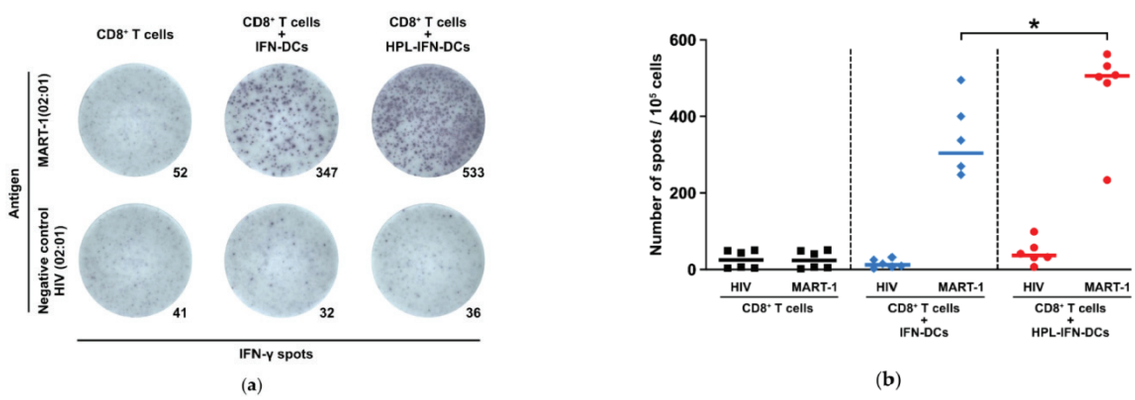
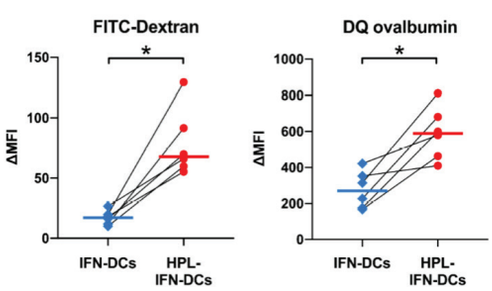


HPL-IFN-DCs for MART-1-specific CTL induction. IFN-DCs or HPL-IFN-DCs were cocultured with autologous T cells at a ratio of E:T = 1:10. (a) Fourteen or 21 days after the start of co-culturing, MART-1-specific CTLs were detected by CD3, CD8, and MART-1 positive gates via flow cytometry (n = 6). These dot plots show a representative example. The percentages in the panels indicate the MART-1 tetramer+ ratio in CD8+ T cells. (b) The graph shows the ratio of MART-1 CTLs co-cultured with either IFN-DCs or HPL-IFN-DCs on days 14 and 21. The bold horizontal bars indicate the median of each parameter (n = 6). * $p < 0.05$. (c) This line graph shows the number of MART-1+ CTLs in the culture period (mean standard deviation). The vertical axis represents the average number of cells (n = 6).



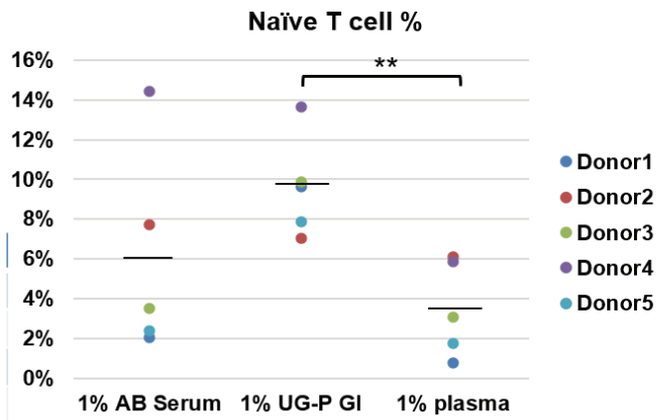
IFN- production in response to MART-1 peptide in CTLs. (a) The representative ELISpot assays highlight IFN--specific spots upon CTL stimulation with MART-1 peptides (ELAGIGILTV) or HIV peptides as the negative control (n = 6). (b) The scatter plots indicate the number of spots for each well. The bold horizontal bars in the graphs indicate the median of each parameter. * $p < 0.05$.

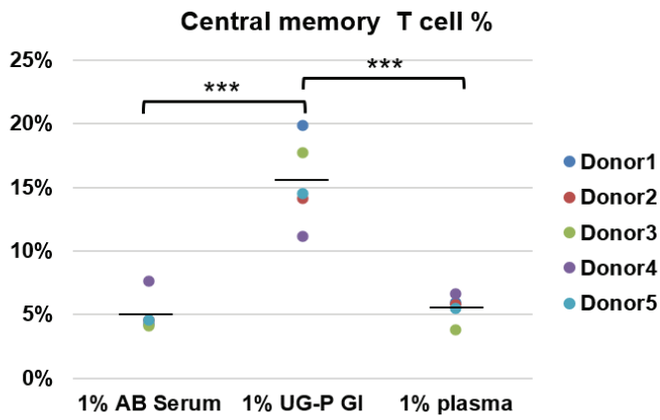


Endocytic and proteolytic activity of HPL-IFN-DCs and IFN-DCs. DCs were incubated with FITC-Dextran to measure antigen endocytosis or DQ ovalbumin to measure proteolytic activity in the maturation cocktail at the time of maturation. * $p < 0.05$.

Immune Cell Culture with UltraGRO™-PURE GI GMP

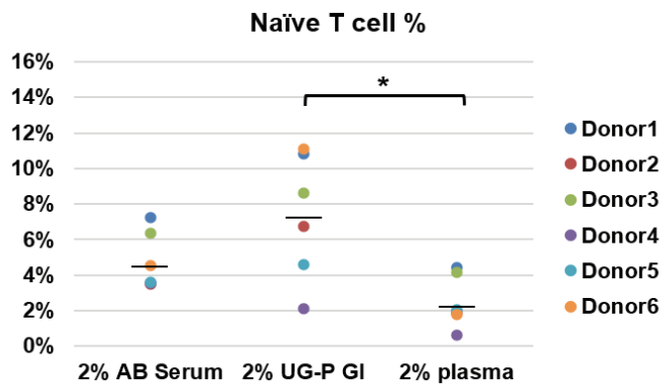
Xeno-free, Gamma Irradiate and Viral Inactivated hPL for Therapeutic T cell Activation

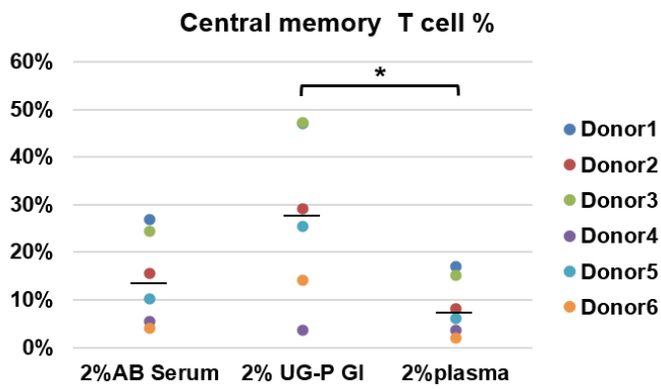




Ave (n = 5)	1% AB serum	1% UG-P GI	1% plasma
Expansion	1321 folds	2610 folds	1708 folds
T cell %	86	82	83
Naïve T %	6.0	9.6	2.5
CM T %	5.0	15.5	6

PBMCs were collected from 5 healthy donors, and T cells were activated by applying a commercial kit from supplier A, followed by the manufacturer's protocol to compare the induction performance with AB serum, UG-P GI, and auto-plasma. The results showed greater T cells with higher population of Naïve and central memory T cells could be obtained by introducing UG-P GI.





Ave (n = 6)	2% AB serum	2% UG-P GI	2% plasma
Expansion	998 folds	2336 folds	1527 folds
T cell %	80	85	78
Naïve T %	4.8	7.4	2.5
CM T %	14.4	27.8	8.6

PBMCs were collected from 6 healthy donors, and T cells were activated by applying a commercial kit from supplier B, followed by the manufacturer's protocol to compare the induction performance with AB serum, UG-P GI, and auto-plasma. The results showed greater T cells with higher population of Naïve and central memory T cells could be obtained by introducing UG-P GI.

Product Number	Product	Bottle Size (mL)
HPCHXCGLI05	UltraGRO™-PURE GI GMP	50
HPCHXCGLI10		100
HPCHXCGLI50		500

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Scalable manufacturing

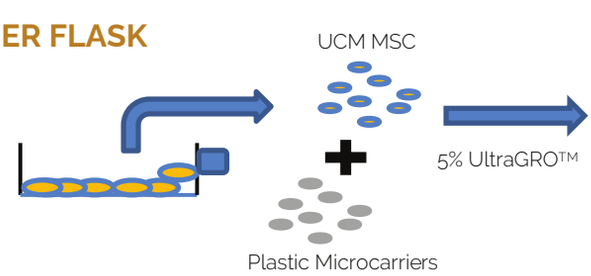
Mesenchymal stem cells (MSC) are multipotent cells with regenerative, multidifferentiation, and immunomodulatory capacities. Expanding MSC for clinical purposes usually requires large cell dose, which demanding efficient cultivation platforms.

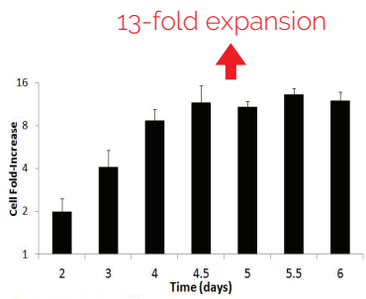
Conventional *in vitro* cell expansion is conducted in static planar culture system. Less efficient space utilization constrains the scale of cell expansion. These limitations impel MSC manufacturing toward three-dimensional culture systems operating under dynamic conditions providing an economic model in respect of consumables, labor, quality controls and facility costs.

The collaboration work of AventaCell and academic institutions have been successfully utilizing human platelet lysate (hPL) to expand MSC in multiple bioreactor systems (de Sure et al., 2017; A. Mizukami de al., 2018; de Sousa Pinto et al., 2019). It demonstrated applicability of hPL for scale-up MSC production in terms of maintaining cell reproducibility, stability and quality.

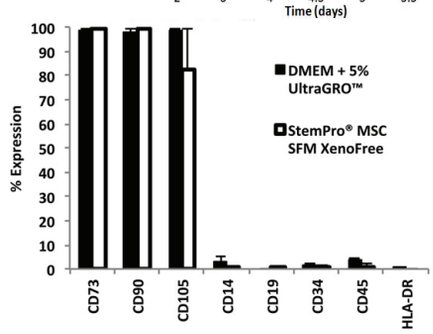
Compatible with Multiple Bioreactors for Seamless for 2D to 3D Scale Up

SPINNER FLASK





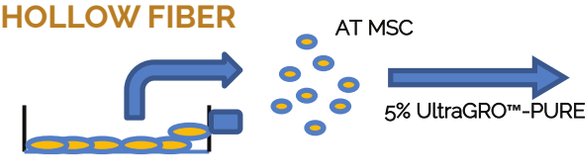
Cell passage	DMEM + 5% UltraGRO™		StemPro® MSC SFM XenoFree	
	Population doublings	Passage duration (days)	Population doublings	Passage duration (days)
P2	5.2 ± 0.45	4.0 ± 0.010	4.4 ± 0.53	5.7 ± 0.33
P3	5.1 ± 0.28	4.3 ± 0.33	3.8 ± 0.12	5.3 ± 0.67
P4	4.6 ± 0.11	4.3 ± 0.33	4.1 ± 0.83	5.3 ± 0.33
P5	4.3 ± 0.45	4.3 ± 0.33	3.6 ± 0.18	5.0 ± 0.58

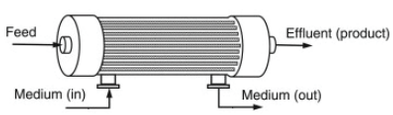


Population doublings of umbilical cord matrix-derived mesenchymal stem/ stromal cells ex vivo expanded under static conditions

J Tissue Eng Regen Med. 11(5):1630-1640 (2017)

QUANTUM HOLLOW FIBER



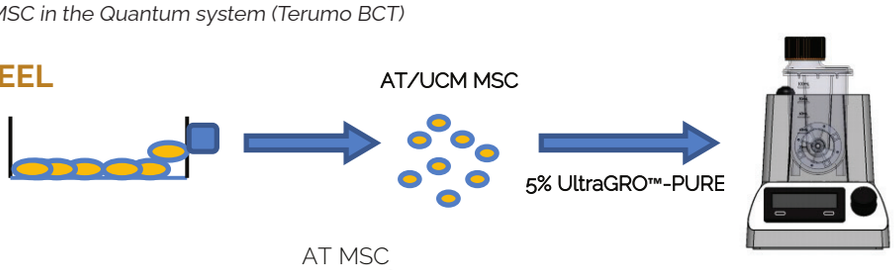


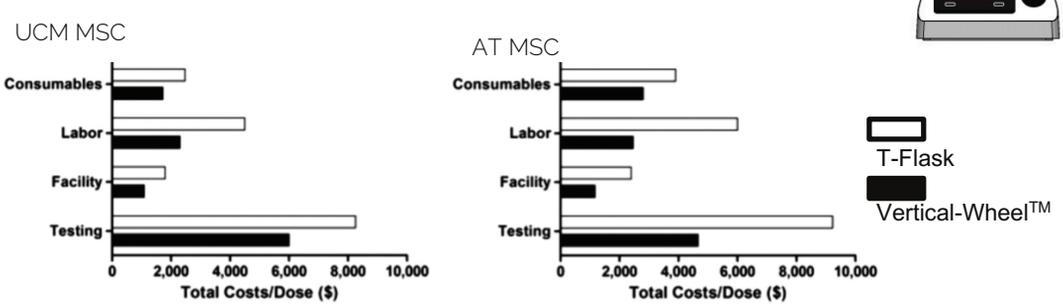
	Our Study	Lenchantuer et al. 2014 [4]	Haack-Sorensen et al. 2016 [5]	Lambrechts et al. 2016 [6]
Mathematics Subject Classification source	Adipose tissue	Bone marrow	Adipose tissue	Periosteum
Culture medium	DMEM + 5% hPL	DMEM + 10% FBS	αMEM + 10% FBS	DMEM + 10% FBS
Cells inoculated (x106 cells)	21	21	21	20
Total cell harvested (x106 cells)	240(±0.42)	208	99(±12)	371
Fold increase	11(±2.0)	9.9	4.7	16
Population doublings	3.4(±0.61)	3.4	2.2±0.19	4.0
Culture duration (days)	5.0	7.0	17±6.0	8.1±0.14
Cell productivity (x106cells/day)	48	29	5.8	45

Summary of culture parameters and results of relevant studies present in the literature for the cultivation of human MSC in the Quantum system (Terumo BCT)

Stem Cell Rev. 14(1):141-143. (2018)

PBS VERTICAL WHEEL





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Your New Options for Closed System Cell Expansion – UltraGRO™-PURE GI Bag Formats




CUSTOM BAG SIZE: MORE EFFICIENT BAG SIZES TO SUIT YOUR SCALE OF PRODUCTION.

Size	Tubing Material	Port 1	Port2	Port 3
50 mL	EVA	1/4" x 5/16" x 10 cm (4") LL male + Cap, pinch clamp	1/4" x 5/16" x 10 cm (4") LL female + Cap, pinch clamp	3/16" x 1/4" x 5 cm (1.97 in.) + septum
250 mL				
500 mL				
1 L	EVA+ Clear C-Flex®374	1/4'' x 7/16'' x 50 cm (20") 3/4" Triclamp, pinch clamp	1/4'' x 7/16'' x 50 cm (20") MPC Male + sealing cap, pinch clamp	1/8" x 1/4" x 50 cm (20"), LL female + needle free sampling port, pinch clamp
2 L	TPE|Clear C-Flex®374	3/8" x 5/8" x 37.5 cm(15") and male MPC+ sealing cap	3/8" x 5/8" x 37.5 cm (15") and male MPC + sealing cap + 3/8" x 5/8" x 30 cm (12") + 1/8" x 1/4" x 50 cm(20") and female Luer® Lock with plug	
4 L				

Connection through welding, MPC quick connectors, and Luer connectors.

Optional tubing size, length, or connectors for fluid transfer can be further customized according to your process demands



ASK US ABOUT CUSTOM BAG SIZES FOR LARGER SCALE-UP PRODUCTION

Product Number	Product	Bag Size (mL)
HPCHXCRLIB05	UltraGRO™-PURE GI – Bag Format	50 ml
HPCHXCRLIB25		100 ml
HPCHXCRLIB50		500ml
HPCHXCRLIB1H		1,000 ml

Product Number	Product	Bag Size (mL)
HPCHXCGLIB05	UltraGRO™-PURE GI – Bag Format (GMP grade)	50 ml
HPCHXCGLIB25		100 ml
HPCHXCGLIB50		500ml
HPCHXCGLIB1H		1,000 ml

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