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

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Introduction

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)[1] 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[2,3], 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[4].

Comparison of Pathogen Reduction Treatment (PRT)

FBS vs UltraGRO™ GI series

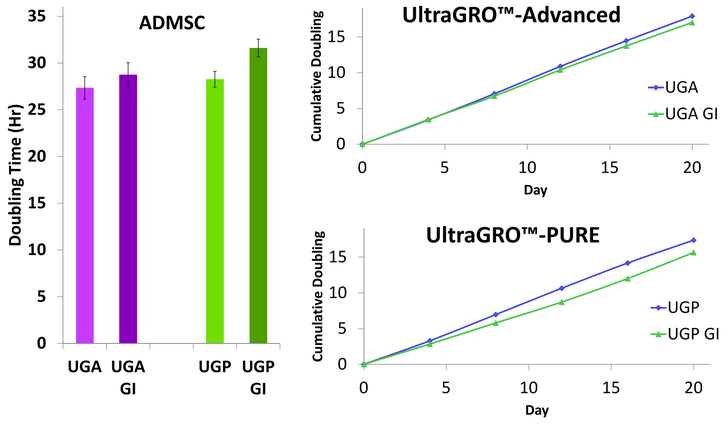
PRT FBS[1]	vs	UltraGRO GI series
0.22μm	**Sterile filtration**	0.22μm
<-10℃	**Finished products storage**	-20℃
Frozen	**Transportation to irradiation plant**	Frozen on dry ice
Gamma	**Irradiation**	Gamma
Cobalt-60	**Radiation source**	Cobalt-60
5-60 kGy (viral inactivation study) 25-40 kGy (typically 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

Viral Clearance Validation

Virus Category	RNA Enveloped	RNA Non-enveloped	DNA Enveloped	DNA 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

Results

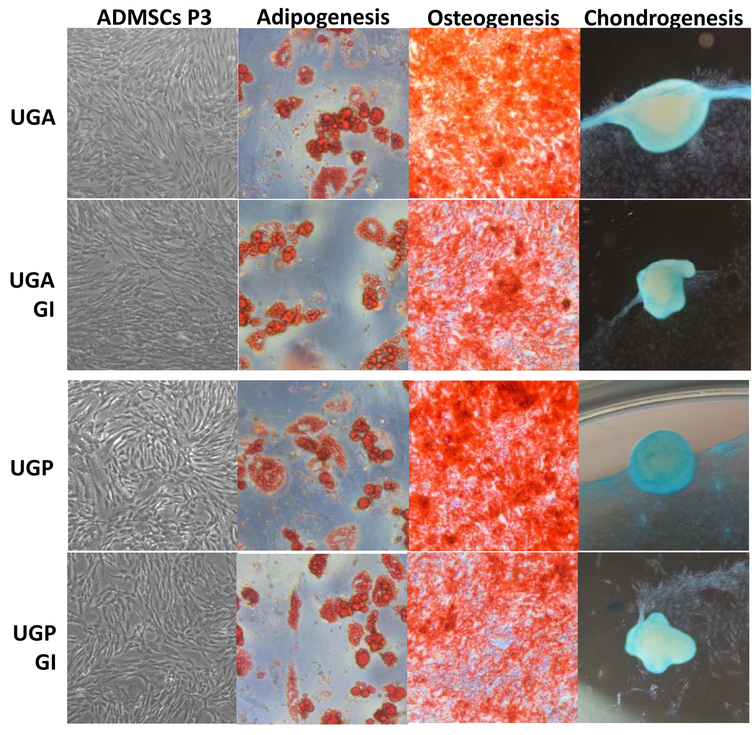
Gamma irradiation has low impact on cell expansion potency of UltraGRO™-Advanced & UltraGRO™-PURE



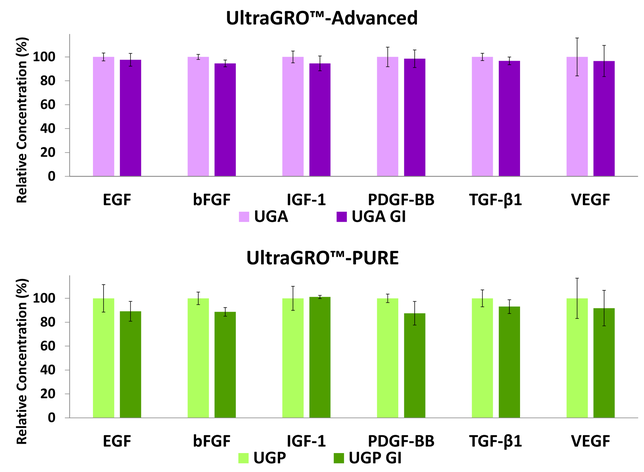
MSCs cultured with gamma irradiated supplements without significant change in immunophenotypes

	Passage 5	CD73	CD90	CD105	CD34	CD45	CD11b	CD79a	HLA-DR
UGA	ADMSC	99.97	99.72	99.51	0.72	0.11	1.19	0.16	1.16
	UCMSC	99.03	99.90	99.94	0.36	0.03	1.85	0.34	1.89
	BMMSC	99.83	100.00	99.00	0.91	0.12	0.51	0.63	1.43
UGA GI	ADMSC	99.95	98.71	99.47	0.49	0.07	1.40	0.16	1.65
	UCMSC	98.12	99.94	99.91	0.25	0.12	1.94	0.51	1.97
	BMMSC	99.98	100.00	98.39	0.86	0.11	0.74	0.18	1.58
UGP	ADMSC	99.95	99.97	99.55	1.07	0.04	0.43	0.30	0.56
	UCMSC	99.08	99.91	99.91	0.11	0.98	1.46	0.31	0.74
	BMMSC	100.00	100.00	99.11	0.08	0.02	0.21	0.20	0.19
UGP GI	ADMSC	99.97	99.88	95.33	0.34	0.40	0.78	0.37	1.65
	UCMSC	95.51	99.98	99.09	0.80	0.31	1.08	1.11	1.97
	BMMSC	99.94	99.50	99.95	0.93	0.15	0.15	0.34	1.45

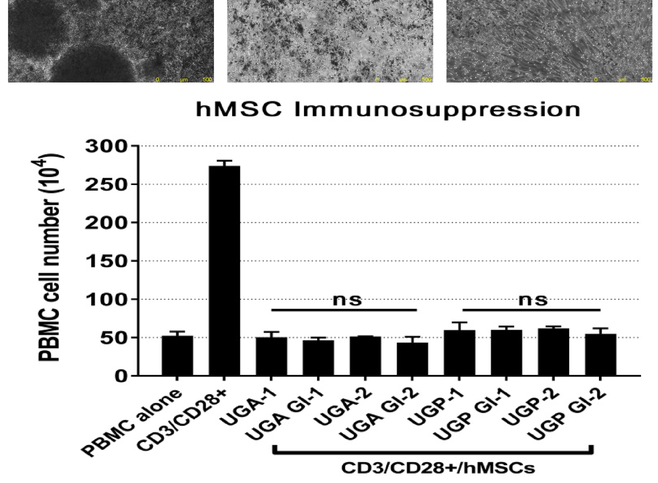
MSCs retain tri-lineage differentiation capability



Growth factors retain comparable levels after gamma irradiation



MSCs retain immunomodulation potency



Conclusions

Gamma irradiation can be considered as a feasible approach for pathogen reduction treatment of pooled human platelet lysate. While gamma irradiation make hPL to be a safer cell culture supplement, we demonstrate gamma irradiated UltraGRO™ GI series products retain the potency to support cell growth and have low impact on key growth factors. UltraGRO™ GI series is an ideal FBS substitute for cell therapy and regenerative medicine applications.

Reference

1. Gamma irradiation of animal sera for inactivation of viruses and mollicutes - Review. Biologicals 2011, 39: 370-377.
2. Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin Q5A (R1)" (ICH, 1999)
3. Revised "Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses" (CPMP/BWP/268/95, European Medicines Agency, 1996)
4. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 4: 315–317.