Viral Inactivation of Pooled Human Platelet Lysate Using Gamma Irradiation

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Advantages of human platelet lysate (hPL)

- Human platelet lysate (hPL) is a xenogeneic-free, growth factor-rich cell culture supplement that can replace fetal bovine serum (FBS) for clinical cell manufacturing.
- Use of hPL over FBS to manufacture cells has the following advantages:
  - Eliminates possibility for patient immunological reactions to animal-derived products.
  - Eliminates possibility for transmission of animal–derived viruses or prions to cell therapy product.
  - Eliminates ethical concerns associated with harvesting of FBS from pregnant cows during slaughter.
  - Promotes faster proliferation of human mesenchymal stromal cells (hMSCs), human adipose-derived stromal cells (hASCs), and a range of other cell types, increasing the efficiency of manufacturing and leading to significant cost savings.

Compass Biomedical's PLUS™ hPL is manufactured using expired, transfusion-grade platelets in accordance with current good manufacturing practices (cGMPs) under an ISO 9001-compliant quality system.

Problem: Potential human virus contamination

- All platelet units used in the manufacturing of PLUS™ hPL are obtained from FDA-registered blood banks and test negative for Hepatitis B, Hepatitis A, HIV Type 1 & 2, Human T-Lymphotropic Virus Type 1 & 2, Zika virus, and syphilis.
- Despite testing, there is a slight risk for contamination with unknown and emerging viruses; regulatory agencies across the globe are beginning to require viral removal or inactivation for blood-derived ancillary materials like hPL.

Solution: Viral Inactivation using gamma irradiation

- A variety of methods exist for destroying viruses in incoming platelet units or in manufactured hPL, but gamma irradiation is the most feasible option.

Method	Mechanism	Target	Feasibility
Gamma irradiation	Ionizing radiation	Manufactured hPL product	Effective against a wide range of viruses; suitable for frozen products
Electron beam irradiation	Ionizing radiation	Manufactured hPL product	Difficult for frozen products (low penetration depth)
Solvent/detergent (S/D)	Disrupts lipid membrane of enveloped viruses	Manufactured hPL product	S/D may alter hPL performance; not effective against non-enveloped viruses
Low pH	Denatures viral proteins	Manufactured hPL product	May denature growth factors and other proteins in hPL
UVA/Amotosalen (Intercept®, Cerus)	Photochemicals crosslink nucleic acids	Incoming platelet units	Photochemicals may alter hPL performance; platelet units not widely available
UVB/Riboflavin (Mirasol®, Terumo)	Photochemicals break nucleic acids	Incoming platelet units	Photochemicals may alter hPL performance; platelet units not widely available
UVC (Theraflex, MacoPharma)	Short-wave UV light blocks transcription	Incoming platelet units	Platelet units not widely available

Goal of this project

- This project sought to confirm the inactivation of model viruses using a standard dose of gamma irradiation and to verify that gamma exposure does not significantly compromise product composition or performance.

Methods

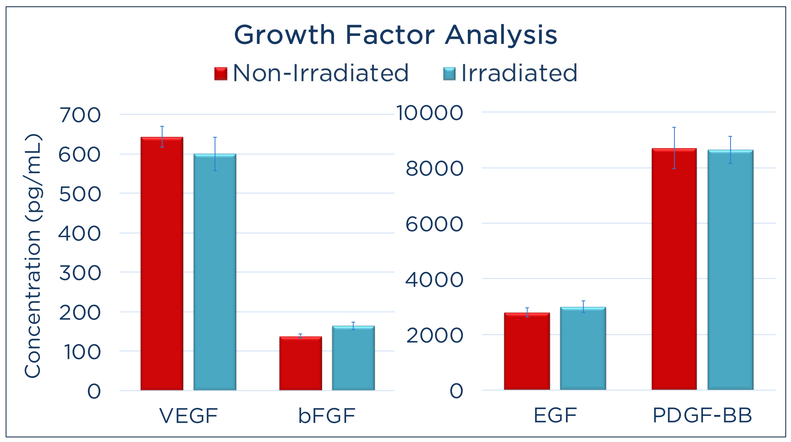
Gamma irradiation: PLUS™ hPL was gamma irradiated at a dose range of 25 - 33 kGy. Product was kept frozen on dry ice throughout shipping and irradiation. A dose mapping study was first performed on surrogate materials to determine the actual delivered dose to the product in the shipping container.

Physicochemical profile: pH, osmolality, and total protein concentration were determined using standard methods. Concentrations of important growth factors (VEGF, FGF-basic, EGF, and PDGF-BB) were determined using ELISA.

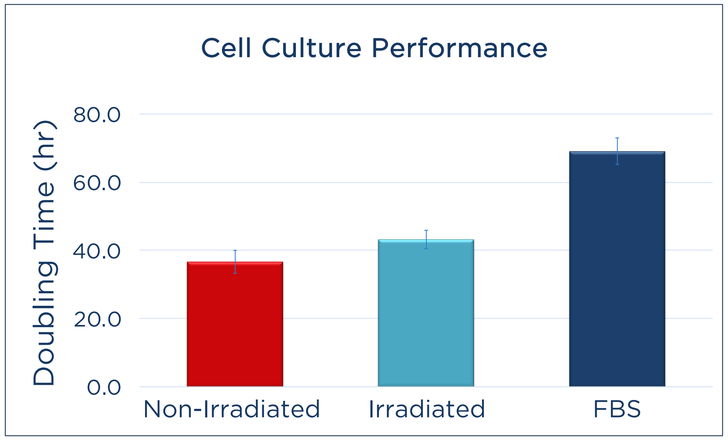
Cell Culture: PLUS™ hPL performance was assessed based on the ability to promote growth of passage 3 bone marrow-derived hMSCs. Cells were cultured in αMEM supplemented with gamma irradiated or non-irradiated PLUS™ hPL or hMSC-qualified fetal bovine serum (FBS) (all at 10% v/v).

Viral inactivation: Viruses were spiked into irradiated and non-irradiated PLUS™ hPL at 5% v/v. Viral titers were determined using plaque assays or tissue culture infective dose ($TCID_{50}$). Viral log_{10} reduction factors (LRFs) were calculated as: LRF = log_{10} (volume*pre-gamma titer/ volume*post-gamma titer)

Gamma irradiation has minimal impact on hPL composition and performance



Growth factor concentrations measured in PLUS™ hPL with and without exposure to gamma irradiation at a dose of 25 – 33 kGy (n = 3 lots).



Doubling time of P3 hMSCs cultured in αMEM supplemented with gamma irradiated or non-irradiated PLUS™ hPL (n = 3 lots) or hMSC-qualified FBS (all at 10% v/v). Cell proliferation was measured using alarmarBlue®.

Gamma irradiation inactivates model viruses

Virus	Family	Genome	Envelope	Size (nm)	Log_{10} Reduction Factor
Encephalomyocarditis virus (EMCV)	Picorna	RNA	No	25-30	5.03
Pseudorabies virus (PRV)	Herpes	DNA	Yes	120-200	>5.49

Log_{10} reduction factors determined for two model viruses spiked into PLUS™ hPL. Values were determined by comparing viral titers in non-irradiated PLUS™ and PLUS™ after gamma irradiation at a dose of 25 – 33 kGy.

Physicochemical Profile

	pH	Osmolality (mOsm/kg)	Total Protein (g/dL)
Non-Irradiated – Lot 1	6.9	323	5.10
Irradiated – Lot 1	7.0	326	5.12
Non-Irradiated – Lot 2	6.8	319	5.21
Irradiated – Lot 2	7.0	340	4.50
Non-Irradiated – Lot 3	7.1	317	4.89
Irradiated – Lot 3	7.1	326	5.05

Physiochemical profile (pH, osmolality, and total protein content) of three different lots of PLUS™ hPL with and without exposure to gamma irradiation at a dose of 25 – 33 kGy.

Conclusions and Future Plans

- Gamma irradiation reduces the risk of potential virus contamination of PLUS™ hPL without significantly impacting product composition or cell culture performance
- Gamma irradiation is a preferred method for viral inactivation of PLUS™ hPL due to:
  - High penetration depth
  - Easily adopted into the GMP manufacturing process
  - Scalable
- Future plans:
  - Quantify inactivation of additional model viruses
  - Assess whether PLUS™ hPL manufacturing process itself removes model viruses

Acknowledgements

- This work was funded by a Phase I SBIR from the National Institute of General Medical Sciences (grant # 1R43GM133224-01)

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