Gamma irradiated human platelet lysate enables safe and efficient cell expansion for cellular therapies

Summary

Transferring cell therapies to the clinic requires large-scale and cost-effective manufacturing platforms for functional cells to be effectively produced at the scale required to meet clinical demand. For mesenchymal stem cells (MSC) and T cell therapies in particular, using human platelet lysate (HPL) in cultures is key to in the safe and efficient expansion of cells ex vivo.

High quality HPL is manufactured from expired platelets collected from healthy human donors. Platelets are obtained through accredited blood banks in the United States and are originally intended for clinical transfusion purposes. Because the platelets were already validated for patient injection, the safety of the material is managed by the US Food & Drug Administration (FDA) and the Association for the Advancement of Blood & Biotherapies (AABB), which set the safety standards for such transfusion products. With the emergence of new infectious diseases, transmission of potential pathogens via blood transfusion or the use of blood derived products in the clinic can be a concern.

This research spotlight summarizes a recent poster presented by Mill Creek Life Sciences demonstrating a high quality HPL that has been treated with gamma irradiation for pathogen reduction and validated for the rapid expansion of MSCs and T cells for therapeutic use. Results show an improved safety profile, cell yield, and cellular phenotype in comparison to other products available in the market.

The Spotlight

Research area: Cell therapy, T cell isolation, T cell culture, T cell expansion, MSC culture, MSC expansion, cell expansion, gene editing, CAR-T, CRISPR, clinical cell culture

Cell type(s): T cells, CAR-T cells, MSC, stem cells

Experiment purpose: Expansion of MSCs and T cells using gamma irradiated human platelet lysate, shows unprecedented levels of product quality and efficiency, which improves the overall safety profile for cell-based products.

Experiment Overview

- **Characterization of gamma irradiated HPL**
- **Biological performance evaluation in MSC cultures**
- **T cell expansion validation with optimized media**

Product Highlights

PLTMax® Human Platelet Lysate, Gamma Irradiated
Originally launched in 2010 and licensed through the Mayo Clinic, PLTMax was the first commercially available human platelet lysate to hit the market. PLTMax requires heparin and is used in several clinical trials worldwide.

PLTGold® Human Platelet Lysate, Gamma Irradiated
PLTGold is a next generation, growth factor rich, xeno-free, heparin-free supplement that is a superior alternative to FBS or human AB serum.

- Superior alternative to FBS
- Validated gamma irradiation (5-virus viral clearance study)
- Produced from clinical-grade platelets
- Available in 27 mL, 100 mL, 500 mL, and 1000 mL bottles
- Drug Master File with the FDA available

Results

Gamma irradiation process validation

Human platelet lysate is treated with gamma irradiation in a validated process to improve viral safety. Dose mapping was initially performed as a distribution study to determine the necessary dose and protocol for effective sterilization. To validate consistency of routine gamma irradiation processing, three 12 liter lots of each bottle size of PLTMax® and PLTGold® human platelet lysate were irradiated using a dose within the accepted range of 25 – 38 kGy and used for further characterization studies. In this research spotlight, the gamma irradiated PLTMax and PLTGold are termed PLTMax-GI and PLTGold-GI, respectively.

Biochemical and molecular characterization of gamma irradiated HPL

Samples from each irradiated product and bottle size were tested for sterility and a variety of chemical and biochemical parameters. These results were then compared with those obtained from non-irradiated samples of the same HPL lot. Statistical analysis showed that there were no significant differences between irradiated and non-irradiated HPL in the following quality parameters: pH, osmolality, and total protein. This result was consistent for both PLTMax-GI and PLTGold-GI for all irradiation rounds and all bottle sizes tested (data not shown).

A panel of 4 growth factors (FGF, PDGF-AB, PDGF-BB, and VEGF) was analyzed for both PLTMax-GI and PLTGold-GI to determine the potential effects of irradiation on growth factors. Statistical analysis determined that no significant differences are seen between bottle sizes, proving that the irradiation dose received by the product was consistent across bottle sizes. However, statistically significant differences were observed between irradiated and non-irradiated HPL for all 4 growth factors, with an average of 10% decrease in growth factor levels observed in the most affected product (data not shown).

Even with this decrease, the detected reduction in growth factor levels observed in PLTMax-GI and PLTGold-GI can be considered very low for an irradiated biologic product. Further evaluation on biological samples was performed to demonstrate continued efficacy of cell culture supplementation.

Viral clearance study on gamma irradiated HPL

In order to demonstrate that the gamma irradiated PLTMax-GI and PLTGold-GI show true pathogen reduction, a viral clearance study was conducted. These results further demonstrate the improved safety of gamma irradiated PLTMax-GI and PLTGold-GI supplements for cultured cells intended for clinical use.

MODEL VIRUS	PLTMAX-GI PLTGOLD-GI	COMPETITOR 1	COMPETITOR 2
Human Immunodeficiency Virus (HIV)	≥5.3 (HIV)	2.9 (HIV)	N/A
Pseudorabies Virus (PRV) or equivalent DNA virus, enveloped	≥6.2 (PRV)	≥5.4 (PRV)	≥4.5 (HSV1)
Bovine Viral Diarrhea Virus (BVDV) or equivaent RNA virus, enveloped	≥6.7 (BVDV)	≥5.1 (BVDV)	≥5.4 (BVDV)
Hepatitis A Virus (HAV) or equivalent RNA virus, non-enveloped	≥6.5 (HAV)	4.9 (EMCV)	≥4.4 (Reo-3)
Porcine Parvovirus (PPV) or equivalent DNA virus, non-envelope	3.0 (PPV)	2.2 (PPV)	4.55 (MMV)

Figure 1. Viral clearance study results. Data shows PLTMax-GI and PLTGold-GI viral clearance data compared to published competitor data for gamma irradiated products. Values show the higher the reduction factor indicated, the more viral inactivation is detected.

Gamma irradiated HPL maintains consistent MSC culture

As expected with a gamma irradiated biologic product, there are some differences between irradiated PLTMax-GI and PLTGold-GI and non-irradiated PLTMax and PLTGold. The effects of gamma irradiation on product performance was evaluated by culturing adipose-derive mesenchymal stem cells (MSCs) with media supplemented with standard PLTMax or PLTMax-GI, and standard PLTGold or PLTGold-GI.

Characteristic differences observed between the products include slightly longer doubling times and lower percentages of confluence at day 5 of culture for the irradiated products compared to the non-irradiated products ($p < 0.0001$ for PLTGold vs PLTGold-GI, and $p = 0.0041$ for PLTMax vs PLTMax-GI). Results were consistent between different rounds of irradiation, with similar effects of irradiation on cell kinetics. While there was a decrease in product performance observed, both PLTMax-GI and PLTGold-GI maintained excellent cell growth rates and confluence in culture.

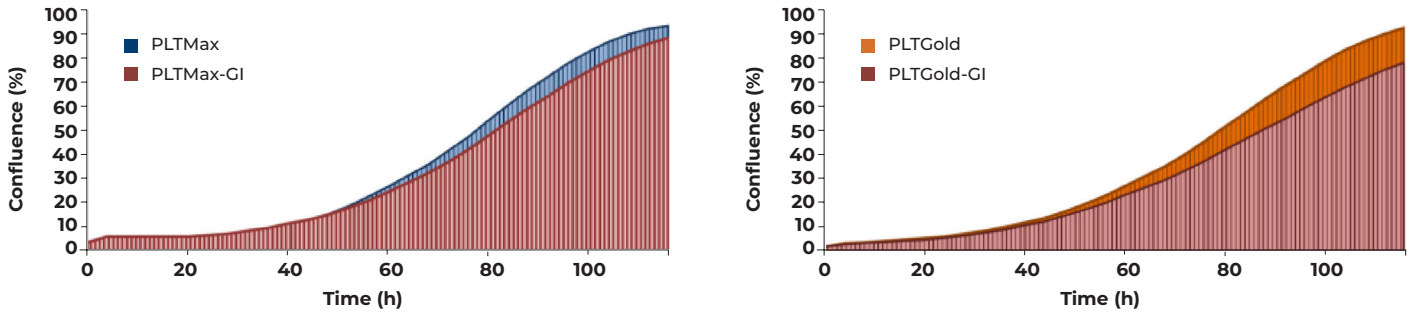

Figure 2. Growth of adipose-derived MSCs in culture media supplemented with either irradiated or non-irradiated HPL. Growth curve of PLTMax vs PLTMax-GI (left) and growth curve of PLTGold vs PLTGold-GI (right) show slight reduction in the potency of the irradiated HPL supplement (6 – 16% reduction observed), while maintaining consistent and strong cell growth in each scenario.

Biological performance evaluation of irradiated HPL

The overall decrease in the potency of the gamma irradiated PLTMax-GI and PLTGold-GI was only 6 – 16% reduction (see Figure 2), as compared to an average of 20% reduction observed in similar commercially available products. Average doubling times obtained with both irradiated and non-irradiated PLTMax and PLTGold supplements are consistently shorter than the equivalent competitor products. In fact, the irradiated of PLTMax-GI and PLTGold-GI still generated cell yields that outperformed even the non-irradiated versions of other commercially available HPL. The shorter doubling times offer a higher cell yield in a smaller timeframe, making PLTMax-GI and PLTGold-GI cost-effective solutions for cell therapy scale-up and biomanufacturing.

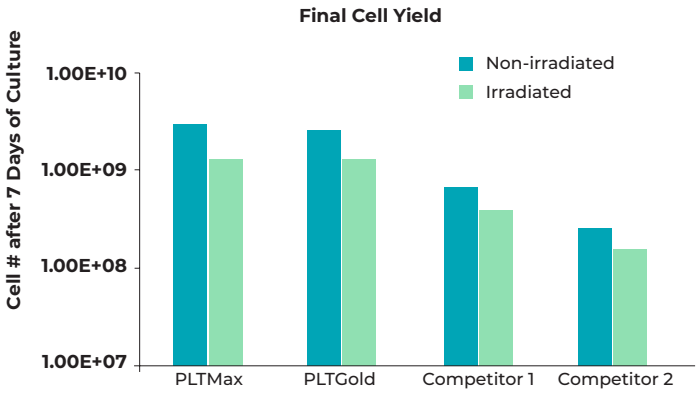

Figure 3. Adipose-derived MSCs expanded in culture medium supplemented with non-irradiated and gamma irradiated HPL. The differences in ultimate cell yield with different HPL after a 10 million cell starting population was cultured over a 7-day period. Cell yields were calculated using internal data and published competitor cell doubling times.

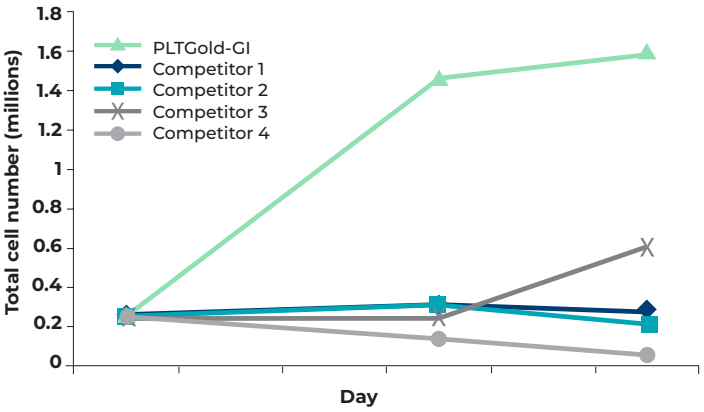

Figure 4. PLTGold-GI outperforms competitor HPL for T cell expansion. Comparison between PLTGold-GI and other commercially available gamma irradiated HPL solutions used as supplements for the expansion of T cells in vitro.

Conclusion

With the continued development of cell-based therapies, PLTMax and PLTGold HPL meet the need for a cGMP, clinical grade culture supplement that is consistent, reliable, and cost-effective for large scale-up applications. PLTMax-GI and PLTGold-GI HPL have been treated with gamma irradiation for pathogen reduction in a process that maintains unprecedented levels of reagent quality and efficiency. PLTMax-GI and PLTGold-GI offer outstanding growth, with further pathogen reduction to improve the safety profile of cell products.

References

1. Alonso-Camino et al. Development of a gamma irradiated human platelet lysate for high efficiency cGMP expansion of therapeutic stem cells. 2021. Mill Creek Life Sciences. Poster.
2. Alonso-Camino et al. Expansion of different types of therapeutic cells using xenogenic free and gamma irradiated human platelet lysate. 2022. Mill Creek Life Sciences. Poster.

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