Development of a gamma irradiated human platelet lysate for high efficiency cGMP expansion of therapeutic stem cells.

Introduction

The effective transfer into the clinic of allogenic cell therapies using Mesenchymal Stem/Stromal Cells (MSCs) depends predominantly on the development of large scale and cost-effective manufacturing platforms that allow production of functional cells at the scale required to meet clinical demand. Human platelet lysate is one key component in these platforms, as it is used for the efficient cGMP expansion of stem cells.

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. Development of platelet lysates with pathogen reduction processes helps to improve the safety profile for these cell-based therapies.

We previously validated the use of INTERCEPT™ Blood System platelets to produce pathogen inactivated PLTMax® and PLTGold®. However, the global availability of INTERCEPT™ treated platelets is limited at this time. For that reason and due to the increasing concern regarding transmission of pathogens through the use of blood derived products to expand therapeutic cells, Mill Creek Life Sciences (MCLS) has developed a more sustainable and long-term process to obtain pathogen reduced human platelet lysate (hPL) while maintaining the properties and quality of our products. Here we describe the development and characterization of PLTMax-GI® and PLTGold-GI® , human platelet lysates that have been treated with gamma irradiation for pathogen reduction in a process that maintains unprecedented levels of product quality and efficiency.

Results

I. Gamma Irradiation of Human Platelet Lysates (hPL) PLTMax® and PLTGold®

After a product dose mapping performed by STERIS Applied Sterilization Technologies, three 12L lots of PLTMax® and PLTGold® were produced in order to obtain three pathogen reduced lots for each product. Each of the lots for a specific product was processed in a separate irradiation batches, which allowed us to determine the variability between irradiation batches. Each lot contained pre-irradiation controls as well as irradiated samples from each commercially available bottle size: 27mL, 100mL and 500mL. Lots were irradiated using a dose within the accepted range of 25-38kG. As a result of irradiation, both products showed changes in appearance: Product maintained the traditional amber color but there was a noticeable increase in turbidity. PLTGold® remained clot free and PLTMax® showed smaller but more abundant clots after irradiation (Figure 1A). Also, microscopic fibers appeared on both products after the irradiation process. However, these fibers proved to not present an issue for cell growth and were able to be eliminated by filtering complete media through a 0.22 µm PES filter. Filtration did not affect product performance.

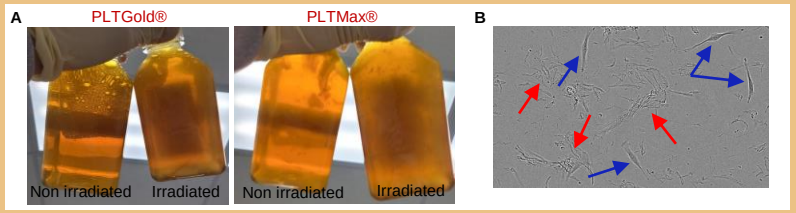


Figure 1. Effects of irradiation on product appearance. A) Appearance of PLTMax® and PLTGold® before and after gamma irradiation. B) Fibers present in hPL after the Gamma Irradiation process marked by red arrows. Cells marked by blue arrows.

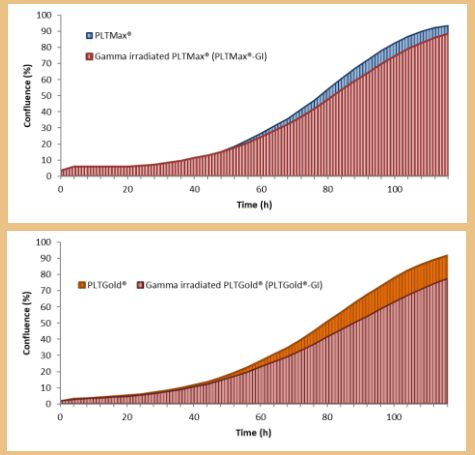
II. Characterization of gamma irradiated hPL : Biochemical and Molecular characterization

At the conclusion of the irradiation process, samples from each bottle size of each lot were tested for sterility as well as for different chemical and biological parameters to compare the results with those obtained from non-irradiated samples of each lot. Statistical analysis showed that there were not significant differences between irradiated and non-irradiated product for parameters like pH, Osmolality or Total Protein. This result was consistent for both PLTMax® and PLTGold® for all irradiation rounds and for all bottle sizes (data not shown). A panel of 4 growth factors (FGF, PDGF-AB, PDGF-BB and VEGF) was analyzed for both products to determine the potential effects of irradiation on growth factors.

Statistical analysis showed that there are not differences between bottle sizes, proving the dose received by the product was the same regardless of bottle size. However, statistically significant differences were found between non-irradiated and irradiated product for all 4 growth factors with an average of a 10% decrease in the worst cases for irradiated product (data not shown). These reduction in growth factor levels can be considered very low for a process like the irradiation of a biologic product.

III. Characterization of gamma irradiated hPL: Biological Characterization

Finally, we evaluated the effects of gamma irradiation on product performance, by growing adipose-derived MSCs with media supplemented with different lots of PLTGold®, PLTMax®, PLTGold®-GI and PLTMax®-GI. As expected after gamma irradiation of a biologic product, there are significant differences between non-irradiated and irradiated product, with higher doubling times and lower percentages of confluence at day 5 of cell culture for the irradiated products *vs* 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. However, we only observed a 6-16% reduction in product potency (Figure 2 A and B) as compared to a 20% reduction observed by competitor products available on the market. Average doubling times obtained with both our non-irradiated and irradiated platelet lysates are lower than those obtained by competitors with their equivalent products. Our gamma irradiated products lower doubling times offering a bigger cell yield in a smaller period of time and therefore are more cost effective (Figure 2C).



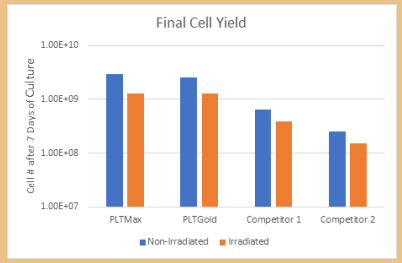


Figure 2. Growth of adipose –derived MSCs in medium supplemented with non-irradiated vs irradiated hPL. A) Growth curve of PLTMax vs PLTMax-GI. B) Growth curve of PLTGold vs PLTGold-GI. C) Differences in ultimate cell yield with different hPL products after starting with 10 million cells and using a 7-day growth period. Cell yields were calculated using internal data and published competitor cell doubling times.

Conclusions

- Our gamma irradiated products offer an added safety measurement with a very low impact on product quality, with only a 6-16% reduction in product potency
- Manufactured under cGMP in large batches (12 liters) to reduce lot-to-lot variation
- Viral Clearance Study underway

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