Viral Inactivation of Pooled Human Platelet Lysate Using Gamma Irradiation

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Adantages 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.

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

Method

- **Gamma irradiation**: PLUS™ hPL was gamma irradiated at a dose rate 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 eMEM 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 (TCID₅₀).
- Viral log₁₀ reduction factors (LRFs) were calculated as: LRF = log₁₀ (volume*pre-gamma liter/volume*post-gamma liter)

Gamma irradiation has minimal impact on hPL composition and performance

**Gamma irradiation inactivates model viruses**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Genome</th>
<th>Envelope</th>
<th>Size (nm)</th>
<th>Log₁₀ Reduction Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalomyocarditis virus (EMCV)</td>
<td>Picorna</td>
<td>RNA</td>
<td>No</td>
<td>25-30</td>
<td>5.03</td>
</tr>
<tr>
<td>Pseudorabies virus (PRV)</td>
<td>Herpes</td>
<td>DNA</td>
<td>Yes</td>
<td>120-200</td>
<td>&gt;5.49</td>
</tr>
</tbody>
</table>

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

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