Characterization of a Pathogen-Reduced Human Platelet Lysate

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BACKGROUND

As more manufactured cellular therapies move into clinical use, there is an increasing call for cell culture media supplements that are of high quality, compatible with different cell types, and not supply-limited. Human platelet lysate (hPL) is one of several supplements available for manufacturing cell products. Derived from multiple platelet units, hPL contains comparable levels of the same types of growth factors and cytokines found in fetal bovine serum (FBS) and human AB serum. Despite the low risk associated with platelet units screened and tested to the same criteria as transfusable blood units, transmission of infectious agents, notably human viruses, remains a consideration for human platelet-derived products such as hPL. Pooling platelet concentrates can improve hPL consistency and performance, but increases the statistical risk of viral contamination.

We have produced a pathogen-reduced hPL (PR HPL), with a process employing electron-beam irradiation (E-beam) to mitigate the viral transmission risk. Characterization of PR HPL is presented here. Viral testing was performed to see if we could achieve pathogen-reduction without significantly affecting hPL composition and hPL functionality as a cell culture supplement.

This study shows that the application of E-beam irradiation for pathogen reduction yields an hPL product that is consistent, preserves cell growth functionality, and reduces the risk of viral transmission.

PURPOSE

The purpose of this study was to evaluate the effect of E-beam irradiation on the characterization and functionality of hPL as a cell culture supplement. The E-beam treated hPL was tested for growth factor levels, ability to support cell growth, and the inactivation of viruses.

METHODS

Figure 1. E-beam mechanism of action

E-beam works on the principle of ionizing radiation: radiation energy capable of detaching at least one electron from an atom or molecule, creating ions.

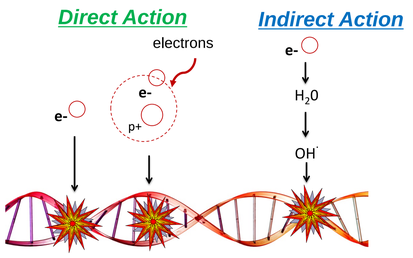
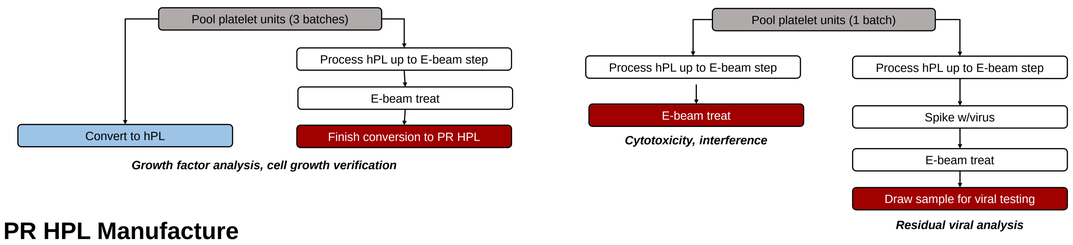


Figure 2. Experimental design



hPL, PR HPL Manufacture

- A total of 240 frozen platelet units (approx. 250mL ea.) were thawed, bags opened, and contents pooled. The pooled platelet volume was equally divided into two separate blood-compatible bags.
- hPL: Platelets were immediately processed to make untreated (no E-beam) hPL.
- PR HPL: Platelets were processed similarly to manufacture hPL, with the exception of the E-beam pathogen-reduction process step. PR HPL was subject to an E-beam irradiation dose in the range of 40-54kGy.

Experimental Methods

- Growth factor levels
  - Growth factors were analyzed by ELISA (R&D Systems®) and a Synergy Neo2 microplate reader (BioTek® Instruments). Growth factors for each treatment and control were analyzed in triplicate.
- Cell growth verification
  - Adipose stem cells (ASCs) and bone marrow MSCs (BM-MSCs) were seeded at 20,000 cells/well in a 12-well plate. Cells were expanded four to five days, then harvested.
  - Total cell counts were performed using a Vi-Cell XR Cell Viability Analyzer (Beckman Coulter®).
- Pathogen-reduction/clearance
  - This feasibility study was performed to evaluate capability of the manufacturing process to remove and/or inactivate virus. A standard viral plaque assay was performed using bovine viral diarrhea virus (BVDV).

Table 1. Model virus

Virus	Family	Genome	Envelope	Size (nm)	Physico-Chemical Resistance
Bovine Viral Diarrhea Virus (BVDV)	Flavi	RNA	Yes	50-70	Medium

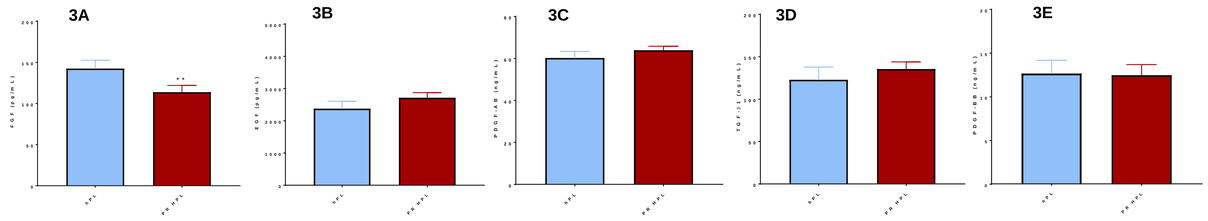
  - Test samples were tested for cytotoxic effects on indicator cells and for interference with viral infectivity.
  - Viral spiking studies were performed using a scaled-down version of the manufacturing process. The spiking virus BVDV was introduced to the sample just prior to application of the E-beam pathogen-reduction step. Residual virus analysis was conducted on the E-beam treated and thawed samples.

Statistical Analysis

- Data are presented as mean ± standard deviation of at least three experiments. Statistical analyses between groups were carried out using a Student's t-test and one-way ANOVA. $p<0.05$ was considered to indicate a statistically significant difference (*=$p<0.05$, **=$p<0.001$).

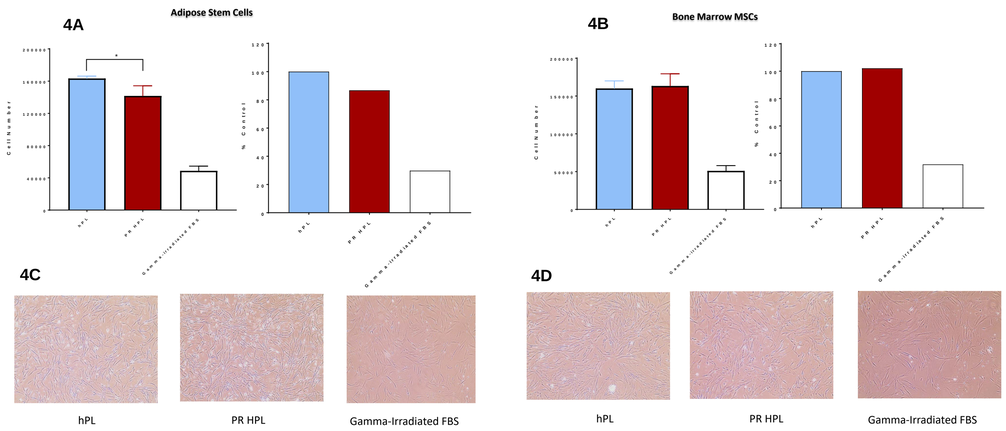
RESULTS

Figure 3. Growth factor analysis



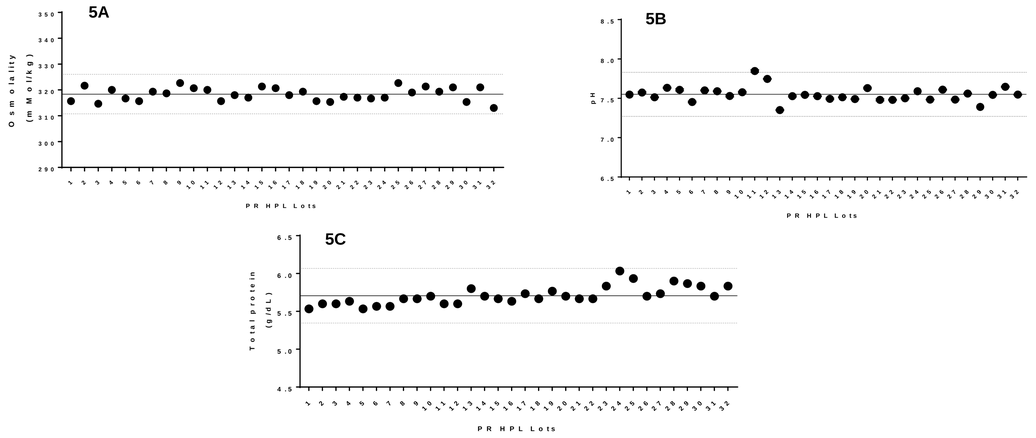
Growth factor profile, hPL vs PR HPL. **A.** Fibroblast growth factor (FGF). **B.** Endothelial Growth Factor (EGF). **C.** Platelet Derived Growth Factor AB (PDGF-AB). **D.** Tissue Growth Factor beta (TGF-β). **E.** Platelet Derived Growth Factor BB (PDGF-BB).

Figure 4. Cell growth verification



Cell proliferation rate comparison. **A.** ASC growth in media supplemented with hPL, PR HPL and gamma-irradiated FBS. Cell growth analysis by cell number and % of control (hPL). **B.** BM-MSC growth results. **C.** Representative images from ASC experiment, comparing cell morphology for the three culture conditions. **D.** Representative images from BM-MSC experiment.

Figure 5. PR HPL biochemical profile



PR HPL lot-to-lot variability (mean ± 3 std dev). **A.** Osmolality. **B.** pH. **C.** Total protein.

Table 2. Pathogen-reduction results

PR Method	Test Group	Model Virus	Log Reduction
E-beam	PR HPL	BVDV	≥ 6.02

Pathogen-reduction for BVDV was greater than or equal to 6.02. This result is expressed as greater than or equal to a log reduction value due to the limit of detection for the viral plaque assay.

CONCLUSION

- At the dose range tested (40-54 kGy), E-beam had minimal impact on hPL performance. ASC cell growth was approx. 85% of control. BM-MSC growth was equivalent to control. Cell morphology was comparable for cells cultured in hPL, PR HPL, and gamma-irradiated FBS.
- E-beam treatment lowered FGF levels, but did not affect the other four growth factors measured: EGF, PDGF-BB, TGFβ, and PDGF-AB.
- Total protein, osmolality, and pH for PR HPL were consistent (less than 10% coefficient of variation) over multiple manufactured batches of PR HPL.
- PR HPL showed a six-log reduction of model enveloped-virus, BVDV, in a viral spiking study.

CONTACT INFORMATION

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