Comparison of a Novel Human Platelet Lysate Formulation to Fetal Bovine Serum for Expansion of Human Cord Blood and Tissue-Derived CD34+ and Mesenchymal Stem Cells.

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Background

There has been rapid growth in the field of cell therapeutics for regenerative medicine applications utilizing cord-derived mesenchymal stem cells (MSCs) and CD34+ progenitor cells. Clinical studies are underway evaluating the therapeutic potential of autologous MSC treatments and have shown clinical benefit with no significant safety issues. Because the use of fetal bovine serum (FBS) has raised safety concerns related to immunogenicity and zoonotic risk, there is a demand for media preparations that are xeno-free which give high cell yields, viability and retention of stem cell phenotypes for cord blood and tissue-based cell therapies. While defined media formulations containing no animal products are available for some cell types, cell expansion and retention of the stem cell phenotype can still be an issue. Human platelet lysate (hPl) preparations have been used as media supplements, but vary widely in their preparation, performance and consistency. We have carried out studies to develop an optimized formulation of hPl as a media supplement.

Cell Growth Assay Results

Fig. 1

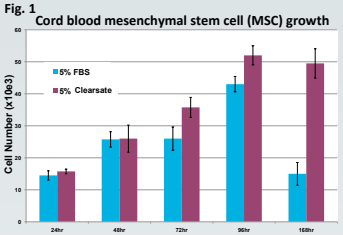


Fig. 2

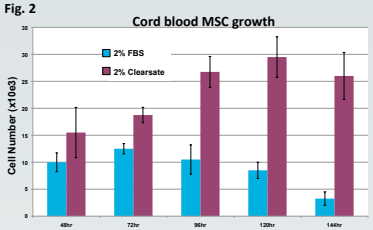


Fig. 3

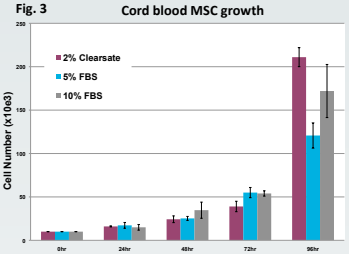


Fig. 4

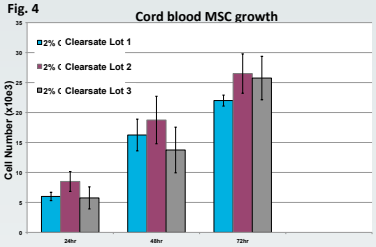


Fig. 5

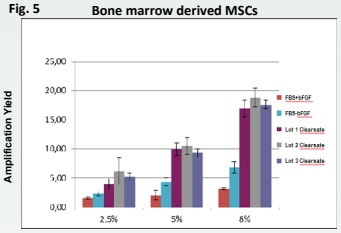
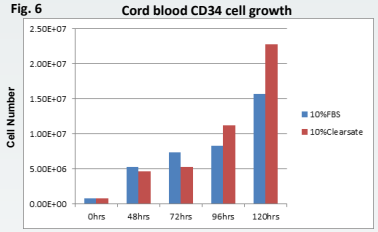


Fig. 6



Materials and Methods

Cell-growth assays utilizing the optimized hPL formulation developed were carried out to characterize the proliferation and viability of MSCs and CD34+ cells isolated from fresh or frozen cord blood and tissue. Parameters evaluated included doubling time, extended expansion time, morphology, CFU and FACs analysis. Comparisons were made with FBS at concentrations ranging from 1-10% in media. Growth assays were conducted for culture durations of up to 168 hours to assess effects on media refeeding of cultures as well.

Results

When MSCs (Figs 1-5) and CD34+ cells (Fig 6) were cultured in the hPL formulation and compared to cultures in FBS, the hPL supplement demonstrated superiority for proliferative capacity and culture maintenance over time with excellent viability compared to FBS. MSCs maintained a fibroblastic morphology and CD34+ cells generated colonies in an in vitro CFU assay. Dose-ranging potency comparisons to FBS with the hPl supplement using MSCs showed shorter doubling times compared to FBS at concentrations of 2%, 5% and 10%. We also evaluated manufacturing lot to lot variability of the hPL supplement on cord blood and bone marrow-derived MSCs and demonstrated excellent consistency in terms of cell growth potency Figs 4,5.

Summary

These data demonstrate that hPl is a promising alternative to FBS for in vitro culture and expansion of cord blood and tissue-derived CD34+ cells and MSCs. Key issues to focus on are consistency in manufacturing and lot to lot potency of the hPl media supplement produced, which have been addressed here and under cGMP manufacturing processes.

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