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Introduction

Human platelet lysate (hPL) arose as a xenogeneic-free alternative to FBS to be used as a supplement for expansion of human mesenchymal Stem Cells (MSCs) used in clinical applications. The use of the first generation hPL involves the addition of heparin to the cell culture media to prevent clotting. Heparin is purified and refined from swine according to cGMP methods and is one of the most widely used drugs in humans. hPL with heparin (PLTMax[®]) has been used all over the world for more than 30 clinical trials. There has been no overt requirement for removal of heparin. However; some quality systems have requested its removal or replacement from cell culture processes in an effort to remove all xenogeneic components.



The chemical (pH and Osmolality) and biochemical analysis (Total protein content) performed for PLTGold[®] showed values comparable to those obtained for PLTMax[®] (Figure 1B).

Adipose derived MSCs grown in media supplemented with PLTGold[®] maintained the characteristic healthy MSC phenotype (Figure 1C), as observed for cells grown in media supplemented with PLTMax[®].

The analysis of growth factors in PLTGold[®] revealed values that fell within the reference range established for PLTMax[®] (Figure 2).



Here we present PLTGold[®], an unfractionated product derived from hPL that does not require the addition of heparin. PLTGold[®] remains clot free and with similar performance as the original PLTMax[®] platelet lysate.

Results

I. Characterization of PLTGold[®]

Mill Creek Life Sciences original product PLTMax[®] has been successfully used in over 30 Phase I, II and III clinical trials for nearly a decade. This product is a xenofree serum alternative, however, heparin is added in the manufacturing process of PLTMax[®] to minimize coagulation. Even with the addition of heparin, precipitates can spontaneously develop over time (Figure 1A, left). Since some cell culture media contain high levels of mediators of coagulation like Ca2+ and Mg2+, the addition of heparin is also required when preparing complete media supplemented with PLTMax.

Figure 2. Comparison between levels of growth factors for PLTMax[®] and PLTGold[®]

II. Cell growth kinetics with PLTGold®

Real time imaging of cell growth using PLTGold[®] as a media supplement to grow adipose-derived MSCs showed increased cell growth kinetics (reduced cell doubling time) compared to cells grown in media supplemented with FBS or Human AB Serum (Figure 3). The performance of PLTGold[®] was comparable to the original hPL, PLTMax[®].



To eliminate the need for heparin addition, Mill Creek Life Sciences has developed PLTGold[®], a step forward in the evolution of hPL. PLTGold[®] contains all the growth factors and proteins necessary for cell growth, but with reduced turbidity and no clot formation (Figure 1A, right).



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Figure 3: Analysis of cell growth using automated cell culture imaging. A) Comparison between cell kinetics of adipose-derived MSC cultured in media supplemented with either PLTMax[®], PLTGold[®], FBS or Human AB Serum. The growth is plotted according to the time in culture and the increase in cell density as measured by confluence. B) Doubling times obtained for cells cultured in media supplemented with different lots of PLTMax[®], PLTGold[®], FBS or Human AB Serum.



Figure 1. Comparison between the first generation platelet lysate PLTMax[®] and PLTGold[®]. A) Appearance of PLTMax[®] (left) *vs* PLTGold[®] (right). B) Comparison between physical and biochemical characteristics for both products. C) Appearance of cells grown in media supplemented with PLTMax[®], PLTGold[®], FBS or Human AB Serum.

C) Representative images of adipose-derived MSCs at day 0 and day 5 of real time monitoring analysis. Cells are false color masked by monitoring instrument software to measure growth.

Conclusions

We report here PLTGold[®], a new Human Platelet Lysate available in Research and Clinical GMP grades with the following characteristics:

- Xenogeneic free
- Heparin-free
- Unfractionated
- Clot-free.
- Contains all the growth factors present in PLTMax[®]
- Performance standards similar or better than the original hPL PLTMax[®].

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