



PLASMA-FREE PLATELET LYSATE PRODUCED IN HUMAN ALBUMIN SOLUTION AS SUPPLEMENT FOR IN VITRO EXPANSION OF MESENCHYMAL STEM CELLS

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INTRODUCTION

Evidence exists indicating that Stem Cell (SC) therapy has the potential to improve organ regeneration in a large spectrum of diseases. Stem cells exist in low numbers in almost all organs. Selected types of SC need to be expanded *ex vivo* to produce the required quantity of SC for clinical application into patients.

In the case of mesenchymal SC (MSC) the majority of expansion procedures are based on the use of fetal bovine serum (FBS). Using FBS harbors the risk of transmission of known and unknown pathogens. FBS use for clinical SC culture is prohibited in Germany and will be prohibited in the EU and US in the near future. Alternatively, limited experience indicates that lysates of bovine and human platelet rich plasma (PRP) may replace FBS in some but not all culture systems. Procedures for producing PRP are highly variable and do not address the risk of disease transmission by human plasma, the risk of anaphylactoid reactions to (repeated) applications of human plasma and of transfusion-related acute lung injury (TRALI). Additional incompatibilities due to blood group-related antibodies (isoagglutinins) present in plasma preparations necessitate the selection of blood group compatible platelet lysate products or of blood group AB plasma devoid of anti-AB antibodies. This dramatically limits the availability of starting material for platelet lysate production because blood group AB comprises less than 5% of all donors and is needed for a variety of medical procedures.

We have invented a new standardized procedure to generate platelet lysates from (1) otherwise discarded buffy coats by (2) pooling multiple units of human buffy coat-derived PRP to reduce variations, (3) removing the plasma to exclude the risk of transmitting isoagglutinins, plasma factors and in particular plasma-related diseases and allergic reactions and (4) substituting the removed plasma by virtually antigen- and antibody-free human albumin solution.

AIM OF EXPERIMENTS

We used human platelet lysate instead of fetal bovine serum (FBS) for *in vitro* expansion of mesenchymal stem cells (MSC) and compared conventional platelet lysate from different donor pools with platelet lysates generated devoid of plasma and plasma protein by lysis in human albumin.

Additionally, we measured cytokines and growth factors in the media on day 0 and d12 of cell culture to detect possible differences in plasma-free platelet lysate compared to conventional platelet lysate.

RESULTS

Figure 1 shows that the mean cell number and the fold increase of initially seeded mesenchymal stem cells (MSC) was highest in culture supplemented with Platelet lysate in human albumin (PL-HA) (4.71×10^6 ; FI 209.27) compared to FBS (US defined) (2.15×10^6 ; FI 95.59), PL (1.81×10^6 ; FI 80.59)

and EBMT/FBS (EU defined) (0.58×10^6 ; FI 26.04). In this particular experiment replacement of plasma by human albumin solution led to a more than twice as high proliferation of MSC as in FBS and PL supplemented culture and to an eight fold as high proliferation rate of MSC stimulated by EBMT/FBS.

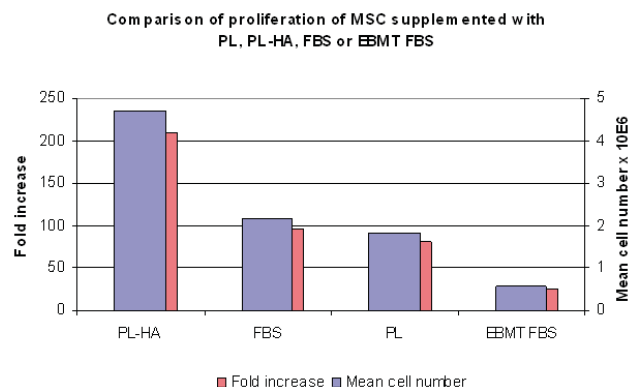


Figure 1

Results of cell numbers and fold increase in MSC cultures with 10% platelet lysate in 5% human albumin (PL-HA), 10% platelet lysate (PL), FBS or EBMT/FBS on day 12. Cells were initially seeded in a density of 50-100 cells/cm².

Results of measured cytokines and growth factors in the supernatants of cultures with PL-HA and PL showed an increase in GM-CSF, IL-6, IL-7, IL-13 and MCP-1 levels during 12 days of culture in PL-HA as well as in PL supplemented cultures. Levels of EGF and PDGF-BB decrease during proliferation of MSC in both culture systems. VEGF production by MSC was more obvious in PL compared to PL-HA which may be of importance for vascular regenerative therapy.

CONCLUSION

We have invented a new standardized procedure to generate platelet lysates by substituting the plasma with virtually antigen- and antibody-free human albumin solution to minimize plasma-associated infectious and to reduce the risk of immunological side effects. We can show that MSC expanded *ex vivo* in medium supplemented with PL-HA proliferate more extensively than with the compared supplements. Kinetics of most cytokines and growth factors during one expansion period of 12 days did not differ significantly from that observed in PL thus indicating the high quality of PL-HA compared to PL.

The use of plasma-free platelet lysate (PL-HA) seems to be a superior alternative to FBS and lysed platelet rich plasma PL for *ex vivo* expansion of SC intended for clinical application.

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COLLABORATION DETAILS

- Collaboration can be in the form of a license agreement or a research cooperation.

POSSIBLE PARTNERS

- blood banks
- stem cell manufacturers
- media manufacturers

DEVELOPMENT STATUS

- patent pending