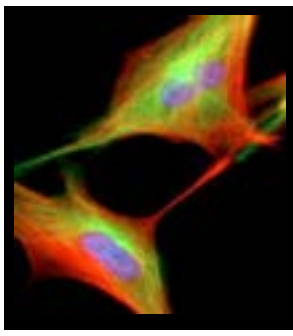

EVALUATION OF STEMULATE™ FOR THE EXPANSION AND CRYOPRESERVATION OF HUMAN MESENCHYMAL STEM CELLS

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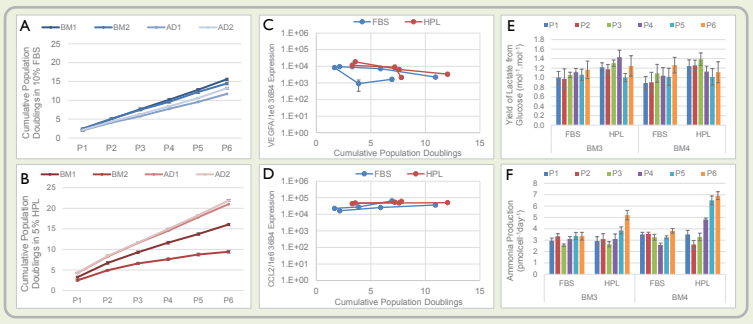
Overview

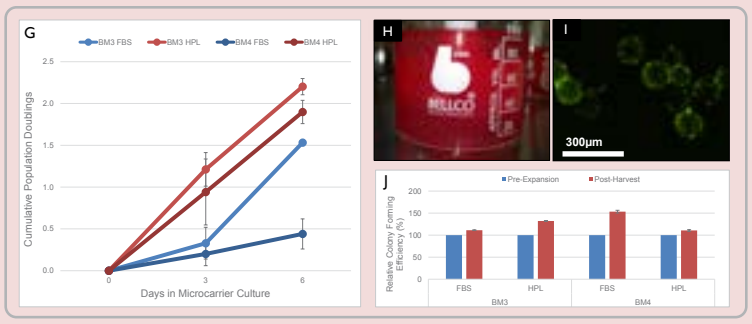
The cost-effective production of AD- & BM-hMSC therapies will require scalable processes with the ability to consistently deliver hMSCs to the clinic. In addition to lot-to-lot variability, there are additional process constraints on the use of bovine-derived serum such as limited supply, potential for pathogen transmission and increasing cost at scale. These considerations are likely to limit the scalability of hMSC manufacturing processes based on bovine-serum and therefore must be addressed. Stemulate™ (human platelet lysate) has been proposed as a viable alternative and this work aims to evaluate this potential.

MONOLAYER

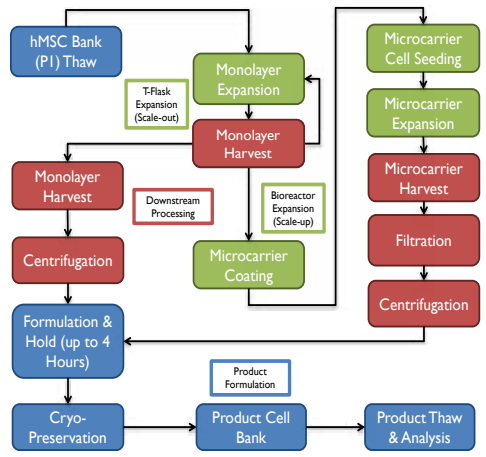


- Adipose-derived (AD-) and bone marrow-derived (BM-) hMSCs have been successfully expanded in DMEM supplemented with 10% bovine serum (FBS) and 5% human platelet lysate (HPL) over six passages (Figure A & B).
- Despite using half the amount of HPL, AD-hMSC expansion was significantly increased compared with FBS (Figure A & B).
- Expression of VEGF has been implicated in the promotion of angiogenesis and has remained present (Figure C).
- CCL2 is an important marker for hMSC immune modulation and has been retained throughout expansion (Figure D).
- Per cell metabolic flux of BM-hMSCs suggests utilisation of similar pathways in FBS and HPL-based culture (Figure E & F).

MICROCARRIER



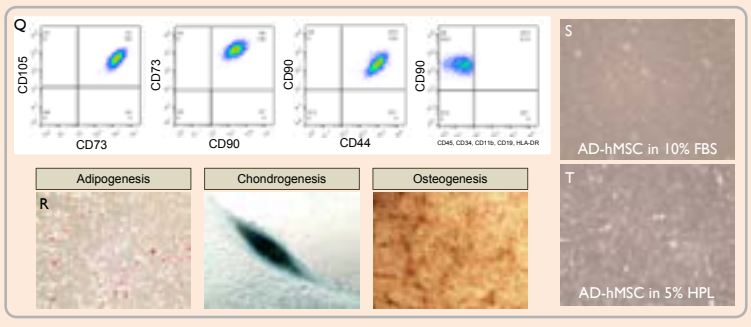
- BM-hMSCs were expanded on non-porous, plastic microcarriers in 100 mL spinner flasks over six days in DMEM supplemented with FBS or HPL.
- Process transfer to a scalable platform is an important step in the development of cost-effective processes.
- HPL increased the expansion rate of both hMSC donors, eliminating the lag phase observed in FBS (Figure G).
- Images show BM-hMSC growth on microcarriers in suspension (Figure H). Live cells stained with Calcein AM fluorophore (Figure I).
- BM-hMSC retained all desired characteristics post microcarrier harvest including CFU potential (Figure J).



Process map for the expansion, harvest, downstream processing and preservation of hMSCs from monolayer & microcarrier processes.

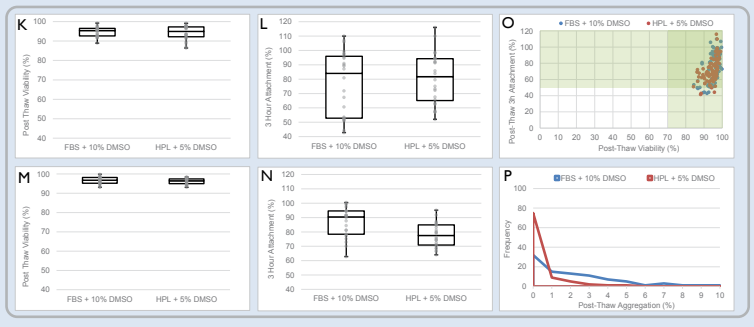
ISCT CRITERIA

- Post-microcarrier and monolayer characterisation of hMSCs cultured in FBS and HPL-based medium demonstrate the maintenance of key characteristics according to the ISCT minimum criteria, namely:
  - Adherence to tissue culture plastic (Figure S & T).
  - Tri-lineage differentiation down osteo-, adipo- & chondrogenic lineages (Figure R).
  - Co-expression of positive immunophenotype markers CD73, 90, 105 & 44 (Figure Q).
  - Co-expression of negative immunophenotype markers CD45, 34, 11b, 19 & HLA-DR (Figure Q).



PRESERVATION

- AD- (Figure K & L) & BM-hMSCs (Figure M & N) have been cryopreserved in HPL + 5% DMSO or FBS + 10% DMSO.
- Reducing DMSO is important for patient safety and as scale rises prolonged holding times in DMSO will damage hMSCs.
- Post-thaw viability (membrane integrity) remained high in both conditions with a low range across all thawing events.
- Attachment efficiency indicates retention of key hMSC function and median value is generally 10% less than viability.
- Figure O shows all thawing events with FDA guideline of >70% post-thaw viability. >50% attachment shows the majority of events retained at least 50% true product yield.
- Figure P shows that HPL + 5% DMSO has a reduced post-thaw aggregation level, important for hMSC clinical delivery.



Conclusion & Perspective

Demonstrating the amenability of Stemulate™ as a replacement for bovine serum in the expansion and cryopreservation of hMSCs provides a step forward in developing scalable manufacturing processes. The increased growth kinetics and batch consistency associated with Stemulate™ combined with the reduced volume required for culture make it a viable alternative to bovine serum for future process development.

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