

ABSTRACT

Our goal is to move human mesenchymal stromal cells derived from Wharton's jelly (WJ-MSCs) into clinical trials. One important step is to generate an SOP to produce GMP-grade MSCs for preclinical evaluation. Here, three commercially prepared serum-free (SF) media and a well-defined 2% serum growth medium (SGM) were compared to determine expansion, phenotypic stability, and multipotency. Xeno free conditions were desired because a single medium is simpler and eliminates exposure to animal products. SF xeno free MSC NutriStem® medium produced by Biological Industries (BI), StemCell Technologies (SC) and Invitrogen (IV) were compared with our SGM. WJ-MSCs were cultured at 10000 cell/cm² in standard conditions of 5% CO₂, 21% oxygen. Additionally, three attachment solutions as recommended by the manufacturers were used for SF conditions (note that expansion in SGM did not require an attachment solution). After initial isolation, WJ-MSCs were split into four media conditions and expanded till passage 5 (P5) and the following parameters were evaluated: expansion, positive and negative surface marker expression, differentiation potential and colony forming unit-fibroblast (CFU-F) assay. WJ-MSCs cultured in MSC NutriStem® medium showed significantly greater proliferation compared to other SF media and to SGM. SF expansion did not impact expression of CD73, CD90, CD105, HLA-ABC (all positive), or CD34, CD45, HLA-DR (all negative). WJ-MSCs differentiated efficiently after expansion in MSC NutriStem®. CFU-F assay revealed no significant difference in colony forming efficiency between MSC NutriStem® and SGM. We conclude that for expansion of WJ-MSCs in SF conditions using MSC NutriStem® and substrate provided optimal cell expansion compared to two other SF formulations and SGM. WJ-MSCs maintained their phenotypic surface marker profile of MSCs and their multipotency as demonstrated by osteocytic, chondrocytic and adipocytic lineage differentiation. Once WJ-MSCs are isolated in MSC NutriStem® medium, preclinical validation testing will begin.

IDENTIFICATION OF OPTIMAL CONDITIONS FOR GENERATING MSCs FOR PRECLINICAL TESTING: COMPARISON OF THREE COMMERCIAL SERUM-FREE MEDIA AND LOW-SERUM GROWTH MEDIUM

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OBJECTIVE

- Compared three commercially available serum-free media to support WJ-MSCs expansion, differentiation and maintenance of CFU-F to our gold-standard medium that contains 2% FBS

INTRODUCTION

- WJ-MSCs are an attractive resource for cell therapy
- We would prefer for clinical evaluations that the cells be cultured in xeno-free conditions
- We compared three commercially available serum-free media for expansion of WJ-MSCs with our gold-standard medium

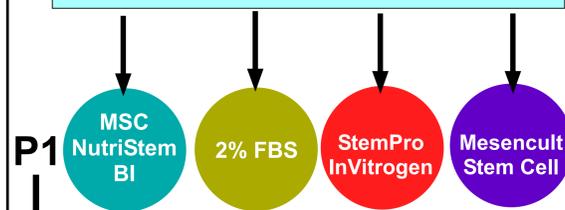
METHODS

- Human WJ-MSCs were obtained from 8 umbilical cords
- The proliferation rate, viability, stemness (estimated from CFU-F), and tri-lineage differentiation capacities were compared in 4 different growth media
- Proliferation and viability were evaluated from P1 to P5, differentiation, flow cytometry and CFU-F assay were performed at P5

METHODS



WJ-MSC primary isolation

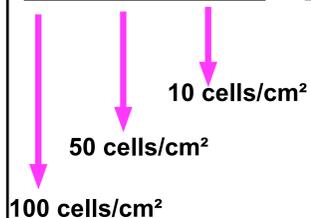


• Trypan blue exclusion test

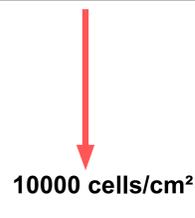
• Proliferation

P5

CFU-F assay

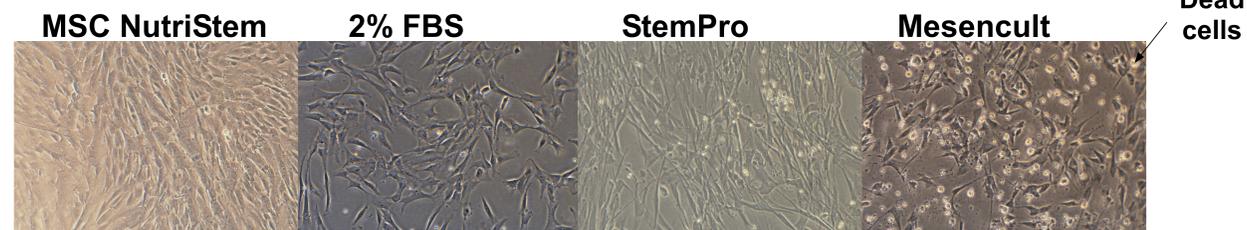


Differentiation

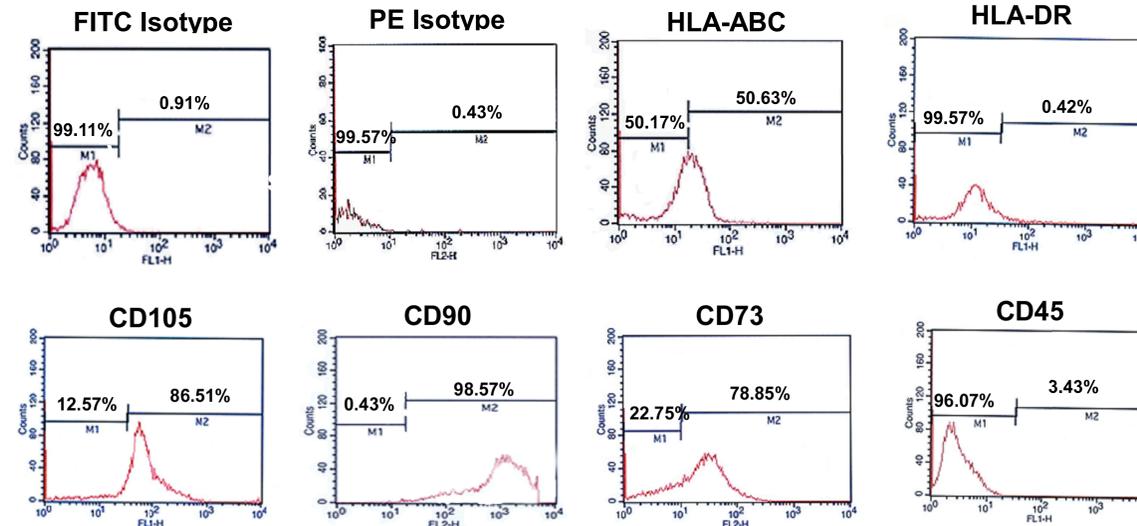


RESULTS

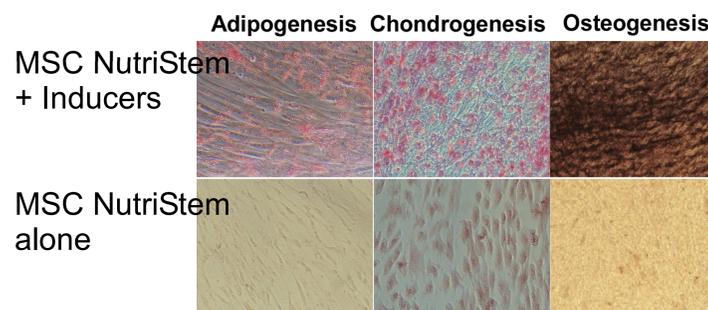
1. Morphology



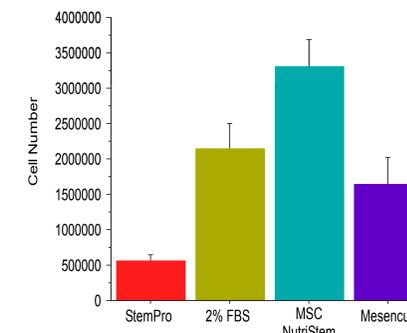
2. Immunophenotyping



3. Tri-lineage differentiation



4. Proliferation



SUMMARY

1. MSC NutriStem outperformed the other media used for WJ-MSC expansion
2. No differences in CFU-F were found between MSC NutriStem and gold-standard medium at passage 5
3. MSC NutriStem and the gold-standard medium supported tri-lineage differentiation with no apparent differences between them.
4. WJ-MSCs stained positively for MSC surface markers from all groups.

CONCLUSION

While more research is required, we conclude that using MSC NutriStem® xeno-free, serum-free medium and substrate provided superior expansion of WJ-MSCs compared to other SF formulations and SGM.

REFERENCES

- López Y *et al.* Evaluating the impact of oxygen concentration and plating density on human Wharton's jelly-derived mesenchymal stroma cells. *TOTERMJ* 4: 2011.
- Seshareddy K *et al.* Method to isolate mesenchymal like cells from Wharton's Jelly of umbilical cord. *Methods Cell Biol* 86: 101-119, 2008.

ACKNOWLEDGMENTS

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 MSC NutriStem is a research product and not yet registered with the FDA.