Effect of BM-HPME® Free Protein on hBM-MSCs in Benchtop Bioreactor with Microcarriers

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INTRODUCTION

Mesenchymal stem cell (MSC)-based therapies have great potential for treating many age-related diseases. When harvested from human bone marrow (hBM), these cells are relatively scarce and thus is is necessary for the cells to be expanded to achieve the necessary numbers needed for any therapeutic application and for many research applications. In order for the potential of MSCs to be unlocked, a strategy must be developed to expand these cells in the most efficient and effective way possible. Here we present a method to expand MSCs in a benchtop bioreactor with microcarriers and quantify the effect of the addition of bone marrow-high performance microenviroment (BM-HPME®) free protein (FP) on this expansion.

METHODS

FIG 1) Extracellular matrix (ECM) or BM-HPME® was produced by hBM-MSCs.

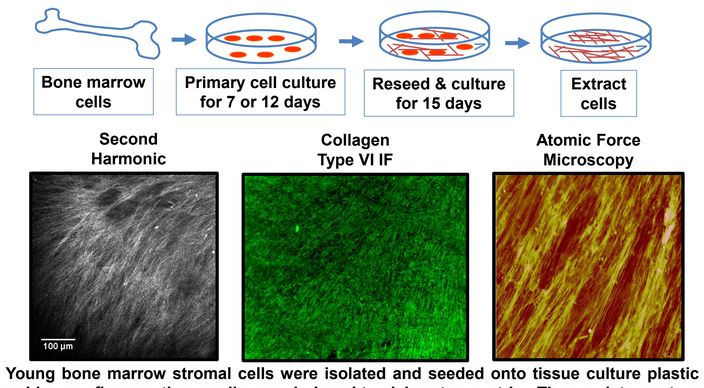
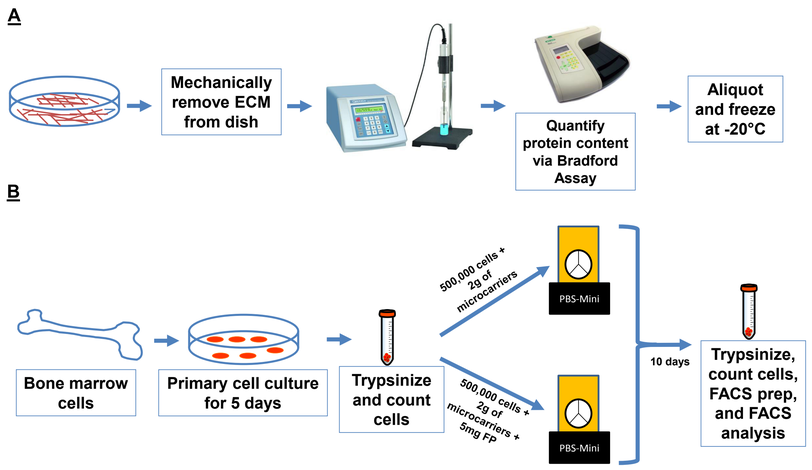


Fig. 1) Young bone marrow stromal cells were isolated and seeded onto tissue culture plastic (TCP). After reaching confluence, those cells were induced to elaborate a matrix. Then, a detergent was used to remove cells, leaving behind a cell-free ECM. Images of the matrix were obtained by secondary harmonic imaging (left), collagen VI staining (center), and atomic force microscopy (right).

FIG 2) Cells were prepared and different bioreactor conditions were prepared, seeded, cultured, counted, and phenotyped.



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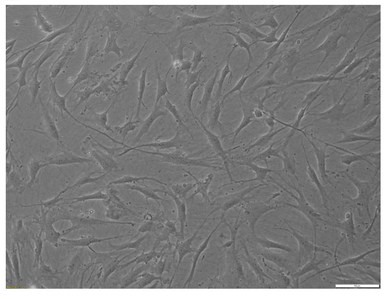

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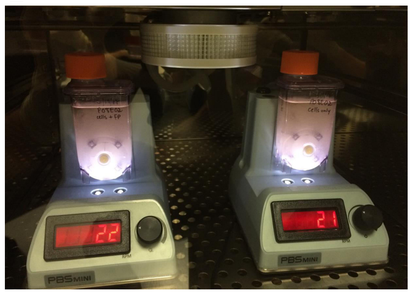

Fig. 2) ECM of BM-HPME® was removed from cell culture dishes to be added to experimental condition in bioreactor, cells were prepared, seeded, cultured, detached, counted, and analyzed via fluorescence-activated cell sorting (FACS). A) BM-HPME® was prepared as shown in FIG 1), ECM proteins (ECMPs) were mechanically removed, sonicated 4 times for 1 minute at 90% amplification with a pulse setting of 1 second on, 1 second off and a 1 minute break between each sonication, the protein concentration was determined via the Bradford Assay, and the ECMP or FP solution was then aliquoted and frozen at -20ºC. B) Bone marrow cells were isolated, cultured to approximately 70-90% confluence in a T150, detached and counted for seeding of bioreactors, seeded at 500,000 cells per condition with the control containing only cells, 2g of microcarriers, and media, and the experimental condition containing cells, microcarriers, media, and 5 mg of free protein. Each bioreactor was cultured in an incubator at 37ºC, 5% CO_2, and ~90% relative humidity while increasing the RPM from 17-25 over the course of the 10 day culture period. Cells were then detached, counted, prepared and phenotyped via FACS to monitor how well the "stemness" of the MSCs was maintained during this expansion process. C) A representative image of the MSCs at ~70% confluence in culture on tissue culture plastic (TCP) during the initial expansion of the cells in preparation for seeding the bioreactors. D) An image of the PBS mini bioreactors in the incubator during culture.

RESULTS

FIG 3) Total cell counts, SSEA4 expression, SSEA4 positive cell counts, and fold change for each experiment (E01-E03)

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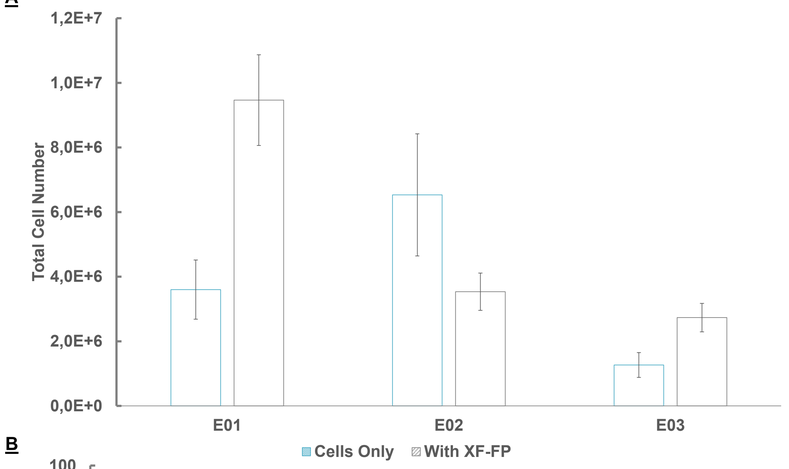

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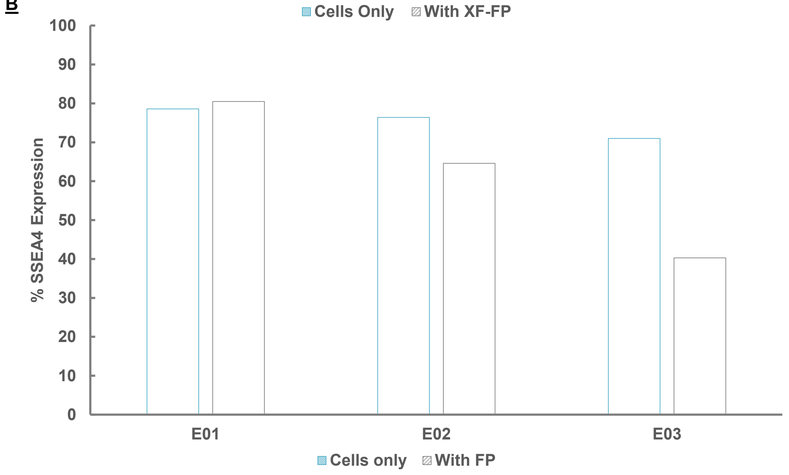

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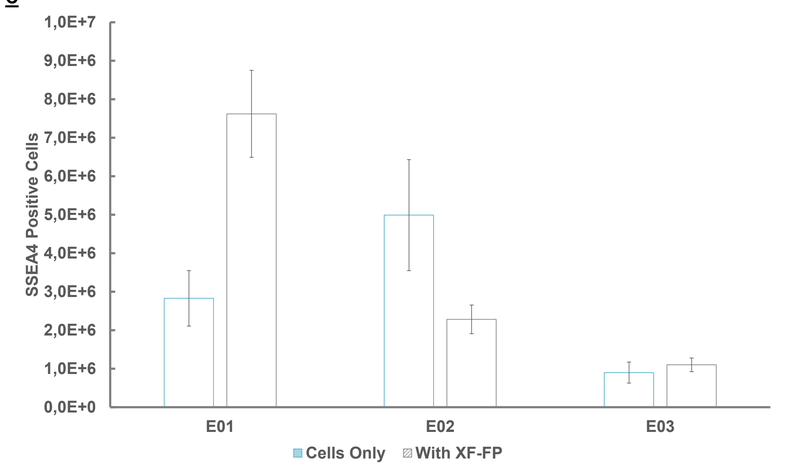

D

	Fold Change for SSEA4 Positive Cells
E01	2.69
E02	0.46
E03	1.22

Fig. 3) Overall, the addition of BM-HPME® FP positively affected total cell proliferation and SSEA4 positive cell proliferation. E02 had the opposite trend as the other two experiments and therefore more experimentation is necessary to understand the underlying mechanism by which the FP is affecting the MSCs. A) Overall, BM-HPME® FP increased MSC proliferation. Cell counts for each experiment were replicated in triplicate and counts were averaged. B) SSEA4 expression varied between experiments. FACS analysis was performed on cells from each experiment. CD90, CD73, and CD105 were used as positive controls with IgG1 as the isotype for these markers. The cells only condition was used as a negative control. SSEA4 was used as the stem cell marker to measure the "stemness" of the cells after the expansion for each condition with IgG3 as the isotype for this marker. In E03, positive controls CD73 and CD105 were unusually low and therefore the SSEA4 expression for this experiment may not be accurate. Further experimentation will be necessary to establish a more consistent trend. C) Overall, BM-HPME® FP increased SSEA4 positive MSC proliferation. Again, E02 is an anomaly and more investigation is necessary to confirm trend. Total SSEA4 positive cells numbers were calculated by multiplying the total cell counts by the % SSEA4 expression. D) Overall, the addition of BM-HPME® FP produced a fold change over 1 for SSEA4 positive cells in this bioreactor expansion method. Fold change was calculated by dividing the total number of SSEA4 positive cells for the experimental condition by the total number of SSEA4 positive cells for the control condition.

Error bars represent standard deviation.

CONCLUSIONS

- MSCs, overall, appeared to respond positively to the addition of the ECMPs (FP) derived from BM-HPME® as demonstrated by the overall increase in proliferation and SSEA4 positive cell proliferation.
- More experimentation would need to be performed in order assess why the second experiment showed the opposite trend as the first and third.
- More consistent results could potentially be achieved if the mechanism by which the FP was affecting the cells (via soluble or adherent factors) was investigated and better understood through additional experimentation.

FUTURE WORK

FIG 4) Plan to investigate whether soluble or adherent factors caused observed increase in proliferation and preliminary data

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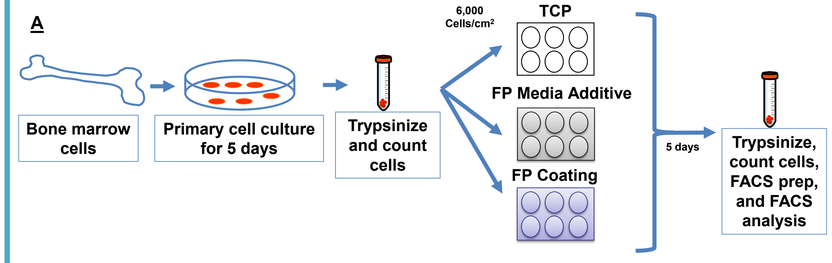

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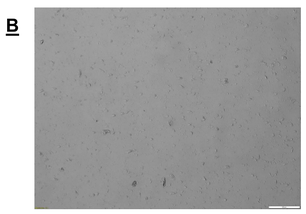

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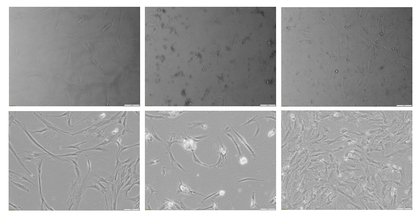

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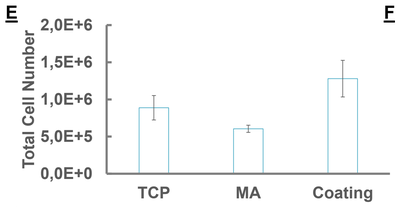

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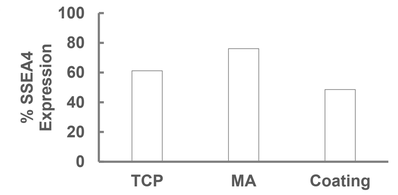

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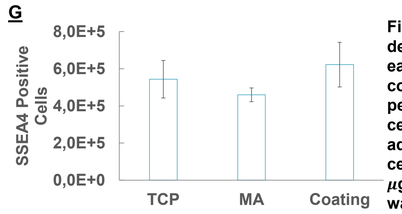

H

	Fold Change for SSEA4 Positive Cells
MA	0.85
Coating	1.14

Fig. 4) MSCs were isolated from hBM, cultured, detached, counted, seeded into 6 well plates for each experimental condition, cultured, detached, counted, and FACS preparation and analysis were performed. FP coating appeared to promote better cell attachment and proliferation. A) For media additive condition, 100 µg/well of FP was added with cell suspension. For FP coating condition, 100 µg/well of FP was added in 1mL of 1X PBS and plate was incubated for 1 hour at 37ºC before seeding. B) Image of FP coating after incubation and aspiration of PBS. C) Images of TCP, MA, and coating conditions (left to right) the day after seeding. D) Images of TCP, MA, and coating on day 5 before detaching. E-H) Cell counts, SSEA4 expression, SSEA4 positive cell number, and SSEA4 fold change were found as previously described.

INTERNSHIP OVERVIEW

- I chose to do an internship because I desired an experience with biomedical engineering in industry to compare and contrast with my experience in academia.
- I accomplished carrying out several projects from beginning to end during my internship.
- This helped me gain professional experience by giving me a better understanding of what industry, specifically working for a startup, looks like.
- I loved my experience at StemBioSys.
- I would definitely recommend that other students do a similar internship.

ACKNOWLEDGEMENTS

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