A New Exosome-depleted Xeno-free Human Platelet Lysate for Therapeutic MSC-derived Secretome Production

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Introduction

Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) play an important role in cell-to-cell communication and have inspired great interest as a new strategy for developing novel therapeutics. Exosomes, one of the subpopulations, comprise various growth factors, cytokines, RNAs, and DNAs. MSC-derived EVs are now one of the promising therapeutic candidates in both regenerative medicine and drug delivery system based on their unique biological properties. However, existing exosomes derived from supplements, fetal bovine serum or human platelet lysate (hPL), will potentially modulate MSC physiology which subsequently affects the quantity and profile of released EVs. In addition, this mixture of EVs can also confound downstream isolation and analysis, leading to misinterpretation of results. Therefore, qualified ancillary materials and controlled culture environment are crucial for MSCs to achieve consistent EV expression profiles batch-to-batch in GMP production.

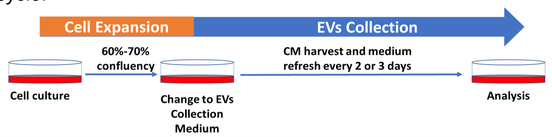
Objectives

In this study, exosome-depleted and xeno-free gamma-irradiated hPL, Exosome-Depleted UltraGRO™-PURE GI (ED UG-P GI) is developed as a novel supplement for MSC-derived EV production. Moreover, pathogen reduction treatment (PRT) by gamma irradiation of the ED UG-P becoming viral inactivated allows the MSC-derived EV production process to comply with regulatory guidance for clinical purposes.

Methods

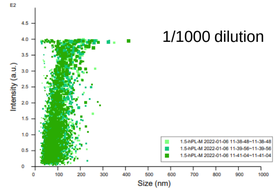
The xeno-free fibrinogen-depleted UltraGRO™-PURE was prepared, and the following exosome depletion was performed by using a tangential-flow-filtration (TFF) system with a hollow fiber module at 500 kDa cut-off. It was conducted at 100 ml/min flow rate with 4000 s^{-1} of shear rate, and 1-10 psi of trans membrane pressure was monitored during the process. Finally, gamma irradiation was the PRT process as the final step of preparation of ED UG-P GI.

When cell confluency reached 60-70% in a petri dish, MSCs were washed with PBS and then cultured with 2% or 5% ED UG-P GI in α-MEM medium for functional study. The conditioned media was harvested every 2-3 days, and the medium was refreshed for a new culture cycle.

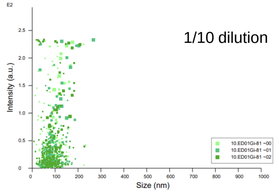


Results

1A



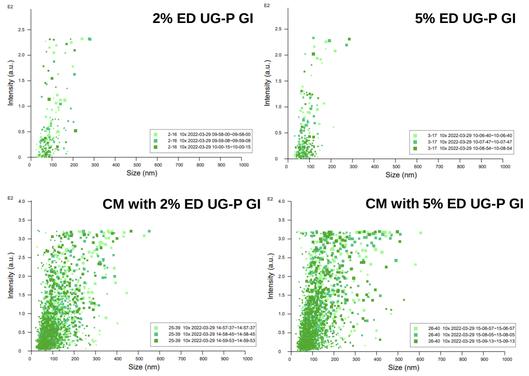
1B



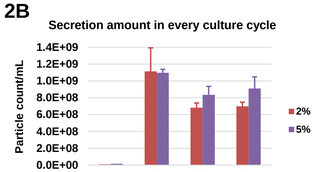
Particle count/mL	Non-depleted hPL	Depleted hPL	Depletion rate
Batch #1	$3.40 \times 10^{11} \pm 2.13 \times 10^{10}$	$5.17 \times 10^{8} \pm 5.25 \times 10^{7}$	99.85%
Batch #2	$2.43 \times 10^{11} \pm 6.63 \times 10^{9}$	$1.40 \times 10^{9} \pm 9.72 \times 10^{7}$	99.42%
Batch #3	$3.35 \times 10^{11} \pm 1.70 \times 10^{10}$	$1.73 \times 10^{9} \pm 2.34 \times 10^{8}$	99.48%
Average	**$3.06 \times 10^{11} \pm 5.46 \times 10^{10}$**	**$1.22 \times 10^{9} \pm 6.27 \times 10^{8}$**	**99.60%**

Fig. 1: Nanoparticles in human platelet lysate were analyzed by Nanoparticle Tracking Analysis (NTA). Nanoparticle size distribution in hPL product (A) before and (B) after showed the significant decrease of the particle signal after the depletion process. Moreover, the outstanding and consistent particle removal from each batch was performed in the study, resulting an average of 99.6% of depletion rate.

2A



2B



2C

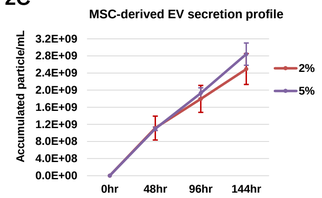
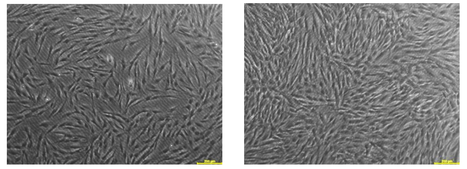
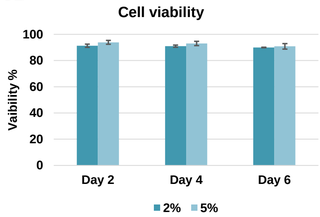


Fig.2: After MSCs reached 60-70% confluency, the culture media was changed to ED UG-P GI supplemented culture media, and the media refresh was performed every 2 days as one culture cycle. (A) Nanoparticle distribution of culture media and conditioned media of MSCs after 2-day culture were analyzed as well as the monitoring of (B) secretion from cells in each culture cycle and (C) the accumulated secretion profile from day 0 to day 6.

3A



3B

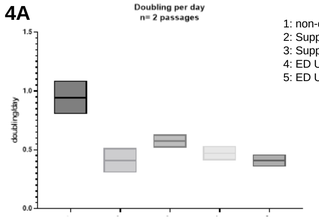


Phenotypic Characterization of MSC

5% ED UG-P GI	Cell viability	CD73	CD90	CD105	CD34
Day 10	87.7%	97.4%	99.4%	99.7%	1.2%
Day 14	91%	97.9%	99.8%	97.6%	1.7%

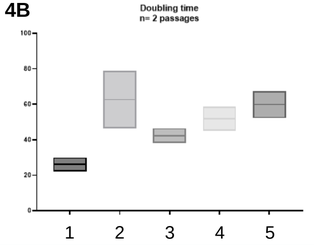
Fig.3: (A) Morphology of the cultured MSCs with ED UG-P GI supplement was observed on day 2 and day 6 with (B) great cell viability (~90%) in both 2% and 5% ED UG-P GI supplemented culture medium. As to long-term culture till day 10 and day 14, MSC remained >87% viable cells and (E) the specific phenotype was not altered throughout the culture period..

4A



1: non-depleted hPL
2: Supplier A SF media
3: Supplier B SF media
4: ED UG-P GI #1
5: ED UG-P GI #2

4B



4C

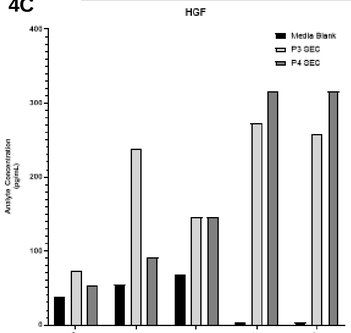
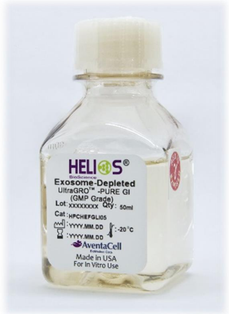


Fig.4: MSCs were first cultured with the non-depleted hPL supplement for 24hr and were washed with PBS before being cultured in media A, media B , and 2 lots of ED UG-P GI supplemented medium. Through 2 passages of cell culture, there was no significant difference between ED UG-P GI group and supplier A or supplier B serum-free media on both (4A) cell doubling per day and (4B) doubling time of MSCs. The secretion was harvested at passage 3 and 4 of MSC for hepatocyte growth factor (HGF) analysis, which indicated using ED UG-P GI supplements can obtain greatest amount of the HGF compared to media A and media B.



Conclusion

We have successfully developed an Exosome-Depleted UltraGRO™-PURE GI supplement with high particle depletion rate and PRT features for MSC-derived EV production. Nanoparticle Tracking Analysis showed >99% of the nanoparticles were successfully removed in our process. Significant secreted particles were found after MSCs were cultured with 2% and 5% ED UG-P GI medium for 2 days. MSC maintained great viability and remained the same specific phenotype throughout 14-day culture period. Cultured MSCs with ED UG-P GI supplemented media showed no significant difference on cell doubling and doubling time compared to other suppliers' serum-free medium. However, the ED UG-P GI cultured MSC secreted the greatest amount of HGF among the groups. Our results indicates that ED UG-P GI is feasible for MSCs to prolong cell activity and to produce therapeutic EVs. Therefore, we believe ED UG-P GI is a promising supplement that can be applied to the massive MSC-derived EVs GMP manufacturing for clinical applications.