A Novel Exosome-Depleted Xeno-Free Human Platelet Lysate for Therapeutic Cell-Derived Extracellular Vesicle Production

Yee-Hsien Lin[1], Han-Tse Lin[1], Cheng-Hung Chou[1], William Milligan[1], Min-Chang Huang[1]
[1]AventaCell BioMedical Corp., Kent, Washington, USA
charles.lin@atcbiomed.com

Introduction

Therapeutic applications of extracellular vesicles (EVs) have become a rising star for new development in the biomedical field. EVs were found to be an important mediator in cell-to-cell communication, playing a major role in disease processes, cancer development, and tissue regeneration. Subcategorizing by size, exosomes comprise thousands of bioactive molecules and genetic information that have inspired great interest in novel therapeutics and diagnostics. Based on their unique biological properties, lipid bilayer exosomes can also be designed as a promising drug delivery system for effective tissue regeneration and cancer treatment. Therefore, a careful choice of methodology is essential to generate cell-derived EVs with good quality in terms of cancer research and therapeutic applications. However, cell culture supplements such as FBS or human platelet lysate (hPL) could potentially modulate target cell physiology due to the inherent supplement-derived EVs. Moreover, results can be misinterpreted and confound the subsequent downstream isolation and analysis. Therefore, qualified ancillary materials and a controlled culture environment are crucial for cells to express consistent EVs batch-to-batch in both research and clinical applications.

Objectives

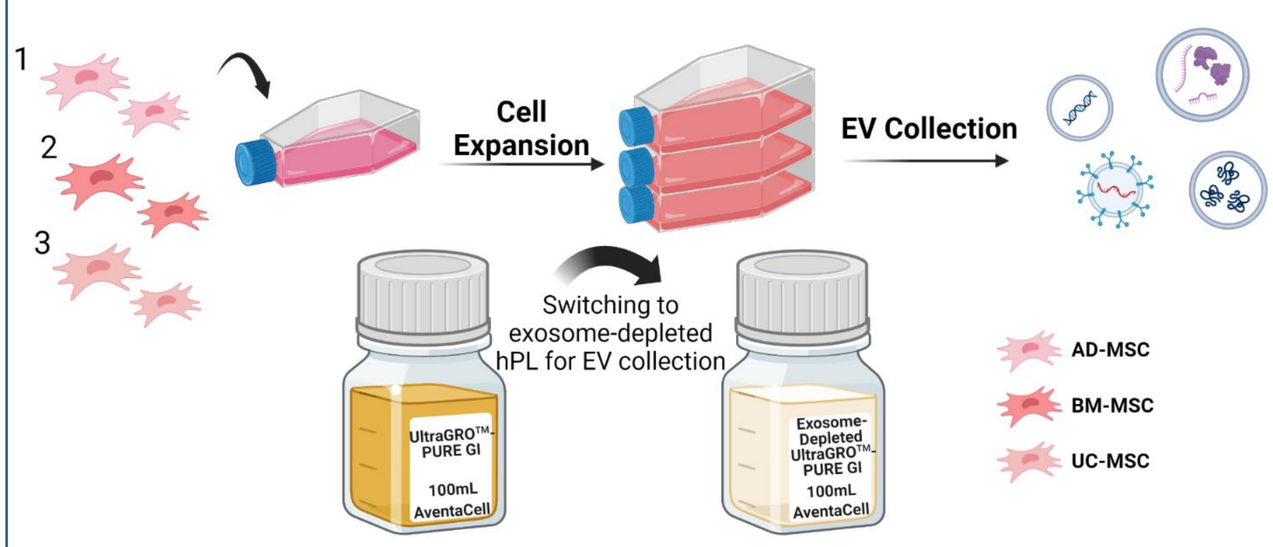
In this study, exosome-depleted and xeno-free gamma-irradiated hPL, Exosome-Depleted UltraGRO™-PURE GI (ED UG-P GI) is developed as a novel cell culture supplement for MSC-derived EV production. Depletion process was performed to minimize the interference. Moreover, pathogen reduction treatment (PRT)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

Methods

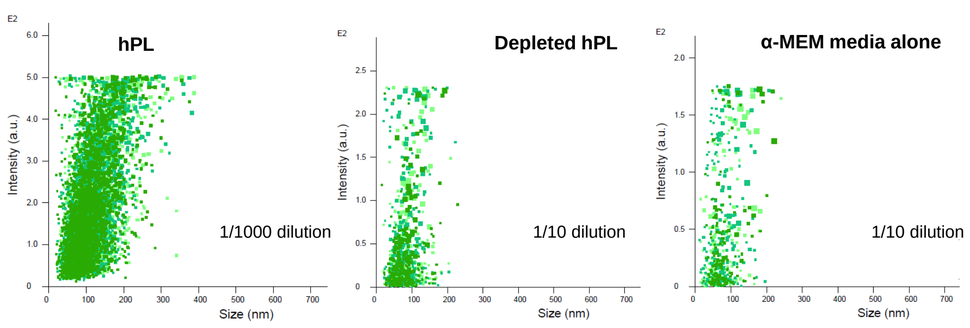
hPL-derived EVs were removed by using a tangential-flow-filtration system, and gamma irradiation was the PRT process as the final step to prepare ED UG-P GI. Particle depletion was examined by Nanoparticle Tracking Analysis (NTA). Adipose-derived MSC (AD-MSC), bone marrow-derived MSC (BM-MSC), and umbilical cord-derived MSC (UC-MSC) were first expanded in 5% UG-P GI supplemented growth medium in a T75 flask separately. When MSC reached 50-60% confluency, cells were rinsed with 1X PBS twice, and then 5% UG-P GI growth medium was replaced with 0-5% ED UG-P GI supplemented α-MEM as collection medium to start the EV production. The conditioned medium was harvested every 2-3 days, and the medium was refreshed for another new EV production cycle. Cell morphology, viability, and MSC-derived EVs were monitored and characterized throughout the culture period.

Results

Experiment Scheme



1A



NTA results	Non-depleted hPL	Depleted hPL	Depletion rate
Particle count/mL	2.73 x10^{11} ± 6.48 x10^{10}	5.2 x 10^{8} – 5.7 x10^{9}	99.07%

Particle in medium	α-MEM media alone	2% ED UG-P GI	5% ED UG-P GI
Particle count/mL	1.93 x10^{8} ± 1.27 x10^{7}	3.51 x10^{8} ± 1.31 x10^{7}	4.32 x10^{8} ± 5.54 x10^{7}

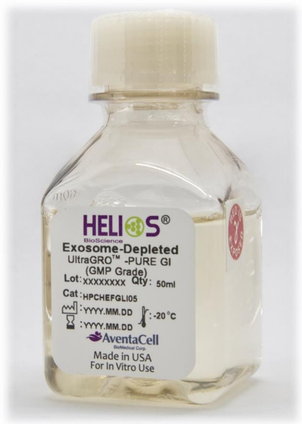
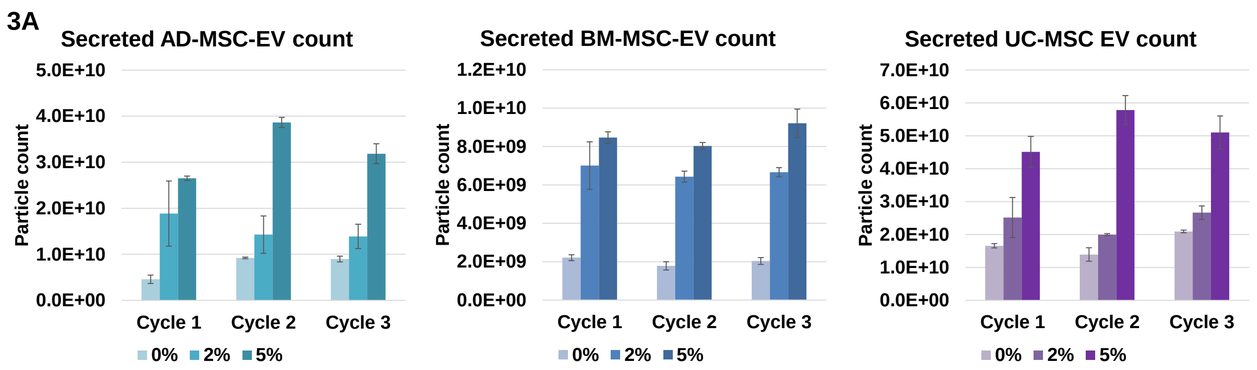
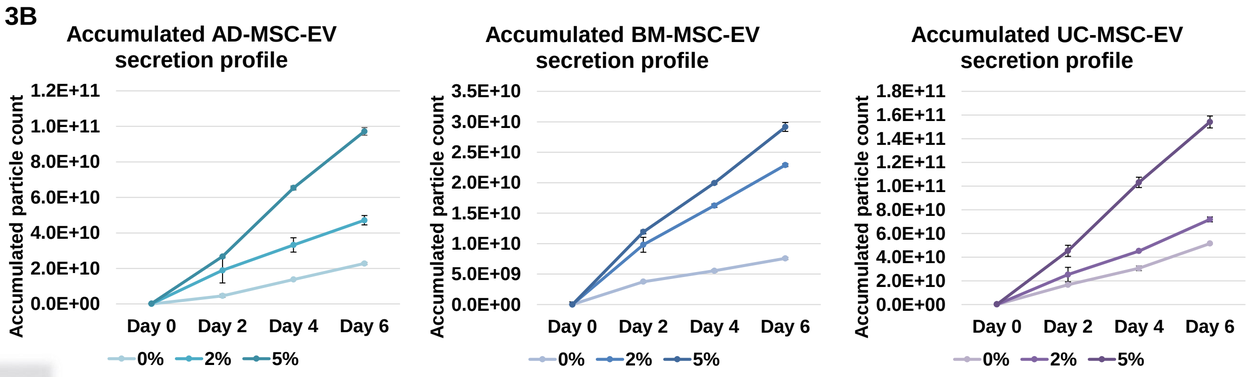


Fig. 1: Nanoparticles were analyzed by NTA. Nanoparticle size distribution in hPL product before and after the depletion process compared to α-MEM basal media alone. Results showed a significant particle removal of the particle signal after the depletion process. Moreover, the outstanding and consistent particle removal from each batch was performed in the study, resulting an average of 99% of depletion rate (n = 5).

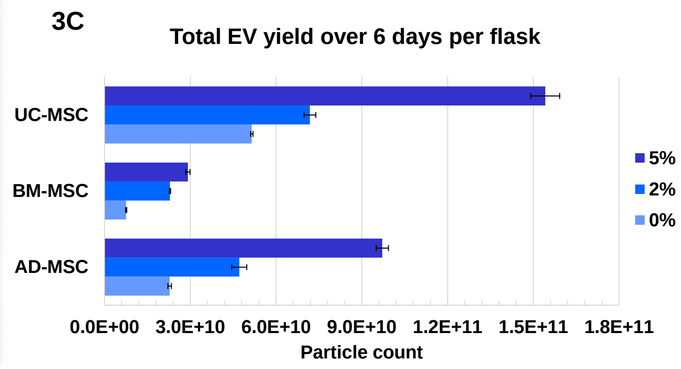
3A



3B



3C



3D

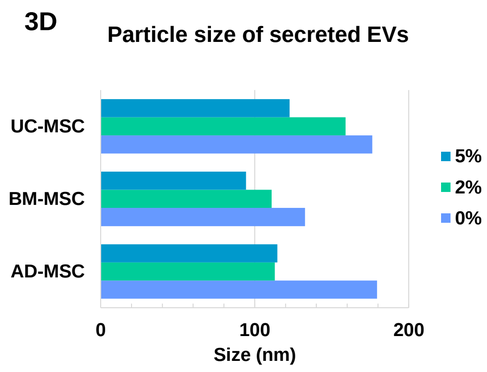


Fig.3: Secreted MSC-derived EVs were collected from each production cycle and were analyzed by NTA. (A) EV secretion profile in each production cycle, (B) accumulated secretion profile throughout the period, (C) total MSC-derived EV yield per T75 flask in 6 days, and (D) particle size of EVs derived from AD-, BM-, and UC-MSCs.

4A

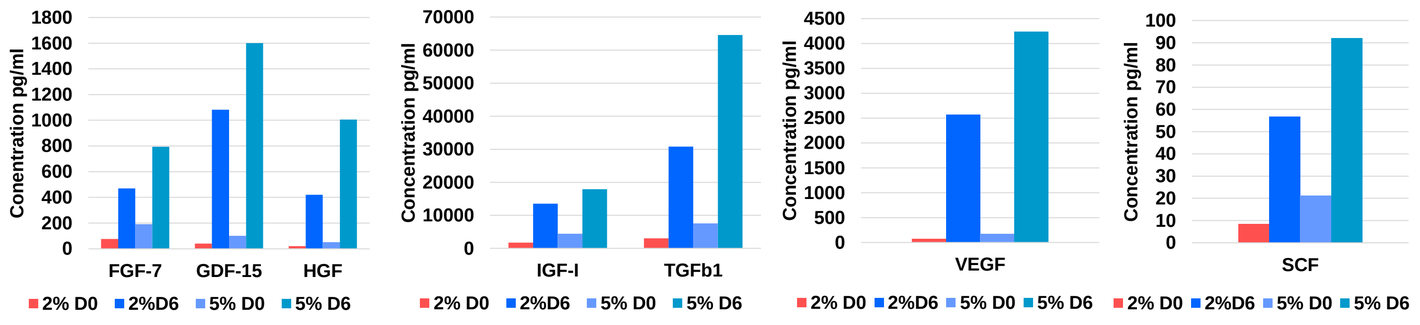
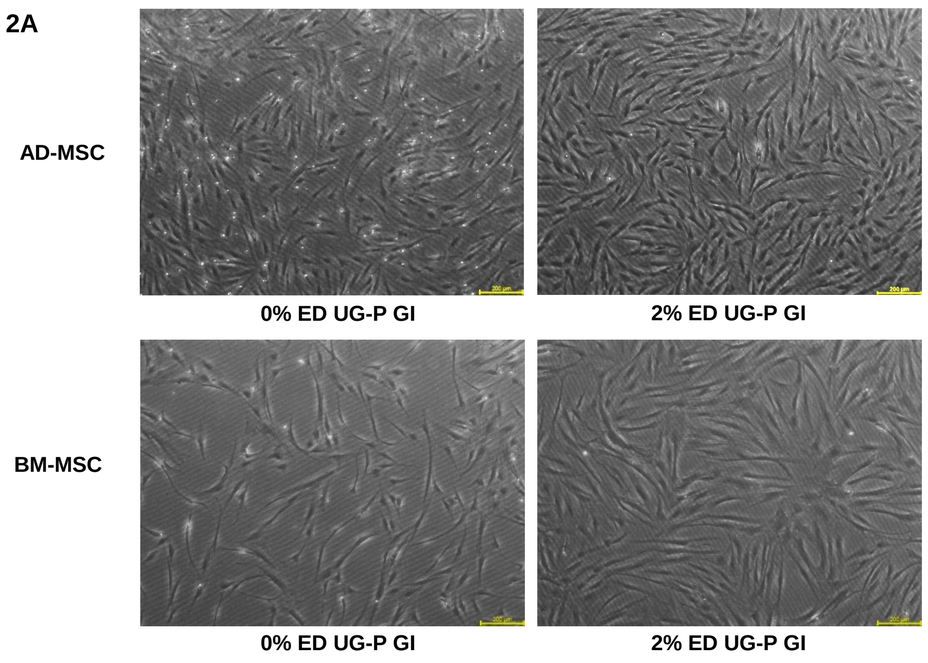


Fig.4: The concentration of growth factors were tested after the conditioned medium was harvested on day 2 and day 6. The target growth factors, FGF-7, GDF-15, HGF, IGF-1, TGFb1, VEGF, and SCF relevant to wound healing process from AD-MSC were significantly enhanced by applying 5% ED UG-P GI medium compared to 2% ED UG-P GI.

2A



2B

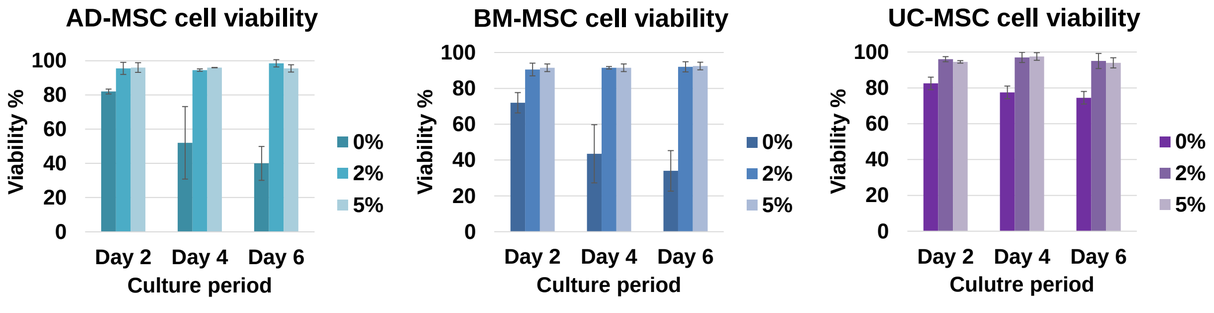


Fig.2: (A) Morphology of the cultured MSCs with 0% (α-MEM alone) or 2% ED UG-P GI supplement was observed on day 2, and (B) cell viability of AD-, BM-, and UC-MSCs were monitored during the culture period. Target MSCs cultured in the presence of ED UG-P GI showed >90% viable with continuous growth compared to α-MEM basal alone which presented a significant drop on day 2.

Summary

- Exosome-Depleted UltraGRO™-PURE GI supplement is designed for MSC-derived EV production with xenogenic-free, high particle depletion rate, and PRT features.
- An average of 99% of the nanoparticles were removed from the original hPL products, leading less than 0.05% of minimal interference when using 5% ED UG-P GI medium for EV production and collection.
- ED UG-P GI can support adipose-, bone marrow-, and umbilical cord-derived MSCs with over 90% of cell viability for long-term EV production compared to α-MEM basal medium alone.
- Billions of EVs can be easily harvested from each T75 culture flask in one production cycle. Also, the therapeutic growth factors for wound healing were characterized from the conditioned medium secreted by the culture MSCs.

Our results suggested that ED UG-P GI is feasible for human MSCs to produce a significant amount of EVs and prolong cell activity. Therefore, ED UG-P GI is a promising hPL-based supplement for therapeutic MSC-derived EV production in both exosome research and GMP manufacturing for clinical applications.