Effect of the culture microenvironment on the production of extracellular vesicles by Wharton's Jelly Mesenchymal Stromal Cells

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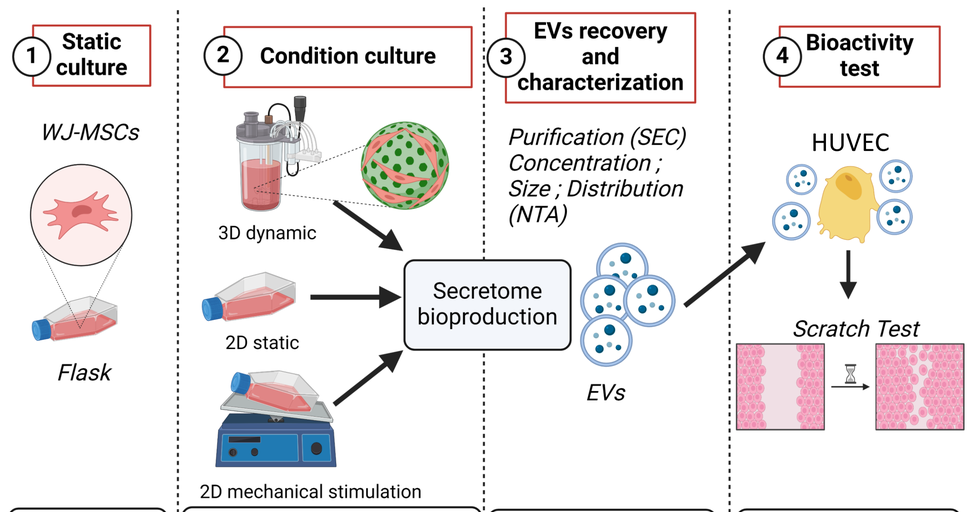
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Context & Objective

Extracellular Vesicles (EVs) are small particles, ranging from 30 to 1000 nm, derived from Wharton's Jelly-derived Mesenchymal Stromal Cells (WJ-MSCs), and have shown significant therapeutic potential in regenerative medicine. Recent research suggests that culture conditions, such as 2D and 3D cultures, mechanical stimulations but as well confluence state impact cells bioproduction applied to WJ-MSCs can significantly influence the quantity and quality of produced EVs [1,2]. Understanding these factors is crucial for optimizing EV production for therapeutic applications.

This work aimed to compare the influence of cell culture microenvironment - physical (confluency rate, flasks vs bioreactors), **biochemical** (i.e. oxygenation, pH) **and mechanical - on the production of EVs to improve the production yield and enhance their therapeutic properties.**

Experimental Approach



SEC = Size Exclusion Chromatography
NTA = Nanoparticle Tracking Analysis

Results

Effect of confluence rate on particle production by WJ-MSCs

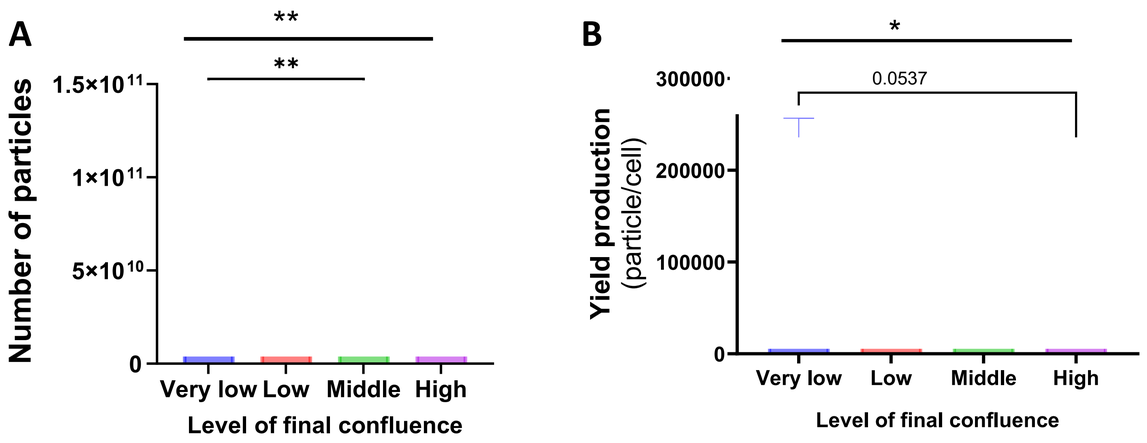


Figure 1: ***Characterisation of particles produced by WJ-MSC derived from 2D monolayer culture*** *(static conditions).* After reaching the targeted confluence, WJ-MSCs were incubated for 48 hours in serum-free medium to collect only EVs produced by the cells. Particles were concentrated with Amicon tube and particles measured in the secretome. (A), Total particle produced and (B) Yield production (n = 4). Error bars represent means ± standard deviation. One-way ANOVA tests were performed, *$p < 0.05$; **$p < 0.01$.

The level of confluence has a significant impact on the number of particle produced per flask (A) and on the particle yield production by WJ-MSC (B). This parameter can be established as a physical parameter of the microenvironment. For therapeutic applications, the quantity of EVs required is high, and the cell culture microenvironment must be controlled. Here , the level of confluence (space occupied by cells) appears to influence the production of particles by WJ-MSCs. However, the mean and mode sizes of the particles are altered by this parameter (Data not shown).

Effect of the cell culture in flasks (static and mechanically stimulated) and in bioreactors (dynamic) on WJ-MSC particle production

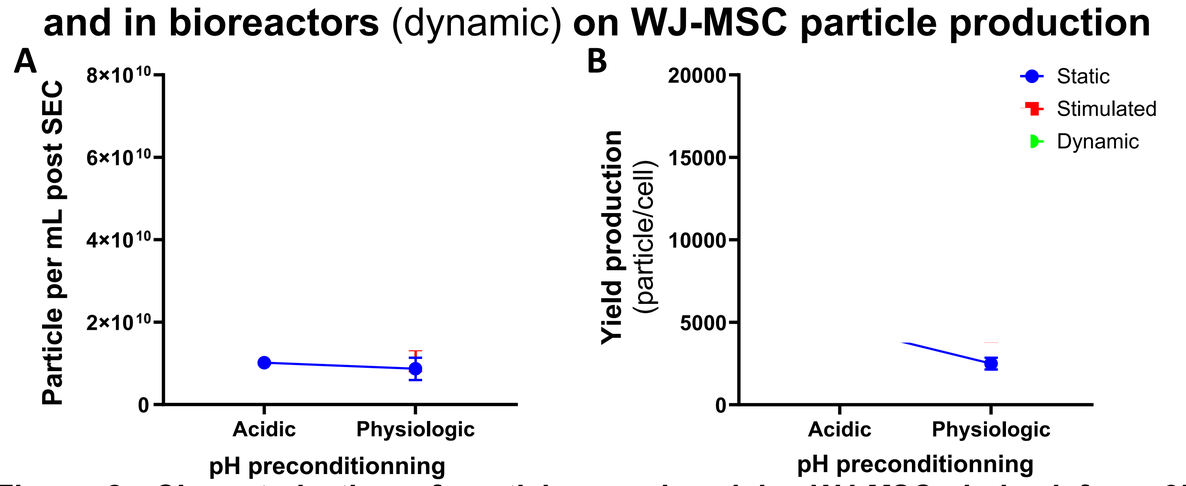


Figure 2: ***Characterisation of particles produced by WJ-MSC derived from 2D monolayer culture*** *(static conditions and mechanical stimulation)* ***and 3D bioreactor culture*** *(dynamic)*. After 2 days of culture and one bout of stimuli (mechanical stimulation and/or acidic pH), WJ-MSCs were incubated for 48 hours in serum-free medium to collect only EVs produced by the cells. Particles were concentrated with Amicon tube and purified by SEC. Then particles were measured in a pure fraction. (A), Total particle produced and (B) Yield production (n = 2-4). Error bars represent means ± *SEM*. Two-way ANOVA tests were performed.

The culture in flask or in bioreactors has not a significant impact on the yield production of particles of this donnor of WJ-MSC. By preconditionning the culture with mechanical stimulations and/or pH changes, it affects the production of Evs by cells. These data are consistent with those found in the literature, where dynamic culture leads to the production of 2.5 to 5 times more EVs per cell in bioreactors and 2 times more EVs per cell in 3D dynamic aggregate culture compared to 2D monolayer culture, with smaller EV sizes [1,2,3].

Effect of oxygenation on the particle production by WJ-MSCs and their functionality

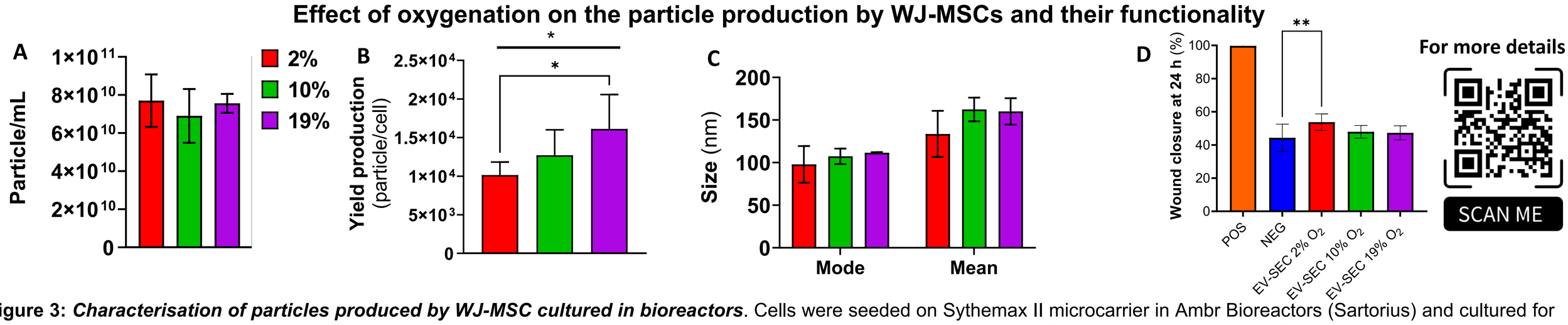


Figure 3: ***Characterisation of particles produced by WJ-MSC cultured in bioreactors***. Cells were seeded on Sythemax II microcarrier in Ambr Bioreactors (Sartorius) and cultured for 7 days in different levels of oxygenation. Then, WJ-MSCs were incubated for 48 hours in serum-free medium to collect only EVs produced by the cells. Particles were concentrated with Amicon tube and purified by SEC. (A), Particle produced by all cells, (B) Yield production, and (C) the size mode and mean were measured with a NTA. (D) The functionality was tested with a wound test on HUVEC and the closure of the gap was measured after 24 hours. (n > 4) Error bars represent means ± SD. One-way ANOVA tests were performed, *$p < 0.05$.

Hypoxia has alter the number of particle produced by WJ-MSC in bioreactors and influenced their bioactivity. In addition, here, we validated the downstream process where pure Evs were obtained (no protein) and with identified tetraspanin markers with a MACSplex (Audoux et al. 2026), which allows us to say that the results on particles can be translated into EVs yield production. The addition of EVs to the HUVEC lead to an increasing migration potential.

Conclusion & Perspectives

In conclusion, our results highlight the impact of cell culture microenvironment on the production of EVs derived from WJ-MSCs. Applying mechanical forces or changing the system of culture (flask vs bioreactors) resulted in a notable increase in EV production. These findings emphasize the importance of integrating controlled and detailed processes into cell culture to improve both the quantity and therapeutic benefits of EVs derived from WJ-MSCs. Furthermore, a more detailed characterization of the EVs produced with these conditions could reveal important functional variations.

References

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For more details on this work or to collaborate on the scale-up of bioprocesses, please contact us

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