

# SHEAR SUSCEPTIBILITY OF HUMAN MESENCHYMAL STEM CELLS INCREASES WITH GENERATION NUMBER: IMPLICATIONS FOR STEM CELL THERAPY SCALE-UP AND MANUFACTURING

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The ability of human mesenchymal stem cells (hMSCs) to withstand shear forces during processing is still debated as there have been reports of hMSCs being damaged during manufacturing. “Shear susceptibility” of suspended hMSCs (harvested from T-flasks) was investigated using a contractional flow device “torture chamber”.<sup>1</sup> Surprisingly, hMSCs were found not to be any more shear susceptible than vero cells (commonly used for vaccine production) provided they are not passaged extensively. (figure 1) Therefore, the number of hMSC doublings before harvesting is limited, which presents a challenge for stem cell manufacturing and scale-up. In order to develop a scale up protocol for hMSCs, we first used HEK293T cells seeded on microcarriers. ANSYS FLUENT was used to model gentle agitation of cells seeded with microcarriers in a “100mm” culture dish to determine the lowest suitable agitation speed. Cells were seeded along with microcarriers in 10mL of media spinner flask with no agitation for 24 hours followed by orbital agitation at 35rpm. HEK293T cells scaled-up using orbital agitation were found to attach and spread to fresh microcarriers more efficiently than cells seeded into an impeller-mixed spinner flask. Transfer from “loaded” microcarriers to fresh microcarriers was found to occur via “contact transfer” or “bridging” between carriers. Hence, orbital agitation is thought to promote this transfer mechanism. For anchorage-dependent hMSCs, attachment efficiency to microcarriers upon seeding plays a significant role in cell production given the apparent passage limitations. Therefore, we expect that when the orbital agitation protocol is used for scale-up of hMSCs, significantly more hMSCs will survive the seeding/attachment process and transfer between microcarriers will be more efficient than in traditional spinner flask microcarrier culture.

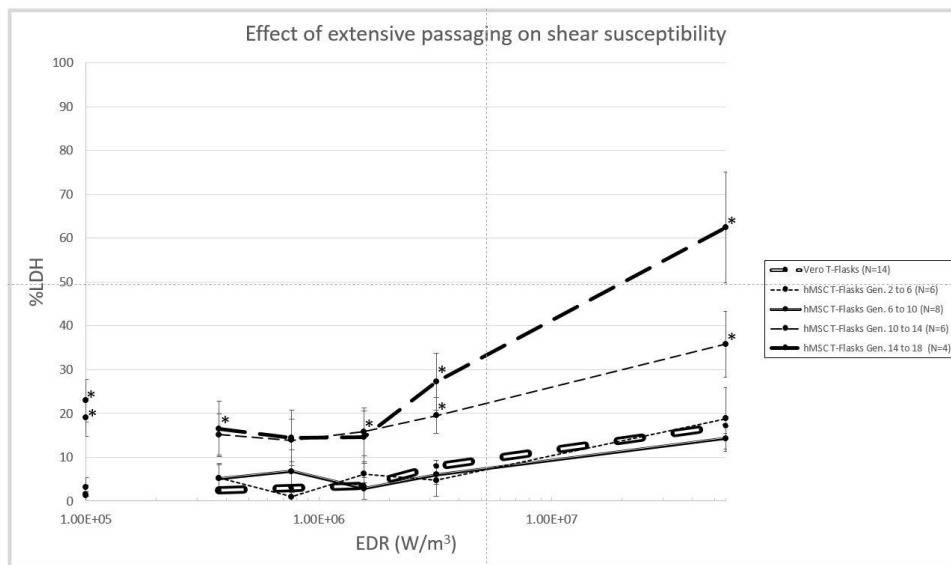


Figure 1 – Vero and hMSC cells (received 18.5 generations old) that had doubled 20.5-24.5, 24.5-28.5, 28.5-32.5 and 32.5 to 36.5 times were exposed to different EDR levels via contractional flow device (10, 20, 30, 40 and 80mL/min). Shear susceptibility was quantified by measuring the amount of LDH released from flowed cells relative to that of lysed cells.

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