

# Supplement 1

## Human Wharton's jelly mesenchymal stromal cells characterization

### Methods

Human Wharton's jelly mesenchymal stromal cells (hWJ-MSCs) were isolated using explant culture, according to the methodology described by Koci et al. [54].

As suggested by Brudet et al [1] and Greenwood et al. [2], cell proliferation was assessed in population doublings (PD). At each passage (P), population doubling time (PDT; 1) PD; (2) was calculated according to these formulas:

$$PDT = (t_2 - t_1) \cdot \frac{\log(2)}{\log\left(\frac{\text{number of cells at } t_2}{\text{number of cells at } t_1}\right)} \quad (1)$$

$$PD = \frac{\log(\text{number of harvested cells}) - \log(\text{number of seeded cells})}{\log 2} \quad (2)$$

Cumulative PD (cPD) was determined as the PD at each passage added to the population doubling of the previous passage (Fig. 1).

hWJ-MSCs in the 3<sup>rd</sup> passage were analyzed for surface marker expression and multipotent differentiation potential.

Flow cytometry was performed using FACS Aria™ (Becton Dickinson, San Jose, California) to confirm the mesenchymal phenotype of hWJ-MSCs. The following antibodies against human antigens CD14, CD34, CD45, CD73, CD90, (Exbio, Czech Republic), CD105 (BioLegend, San Diego, CA, USA), HLA ABC, and HLA-DR (Pharmingen, USA) were tested. Data analysis was performed using BD FASCDiVa software.

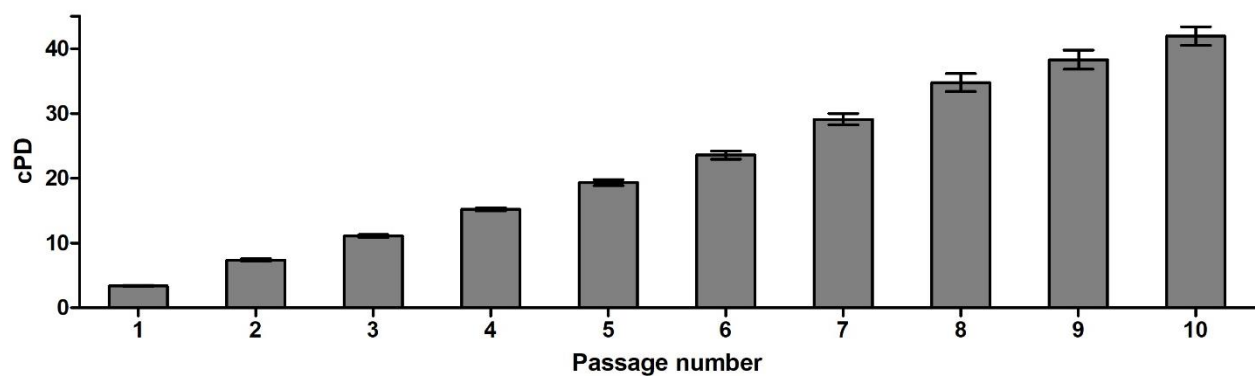
hWJ-MSCs were differentiated into adipocytes, osteoblasts and chondrocytes to show their multilineage potential as described in [3]. Cell differentiation into various lineages was confirmed by specific staining and observed under a light or fluorescent microscope.

### Results

The average PDT time of hWJ-MSCs during the study period, from P1 to P10, was  $33.94 \pm 2.26$  hours. hWJ-MSCs maintained similar PD between passages (Fig 1), which confirms their relative long-term stability and low senescence.

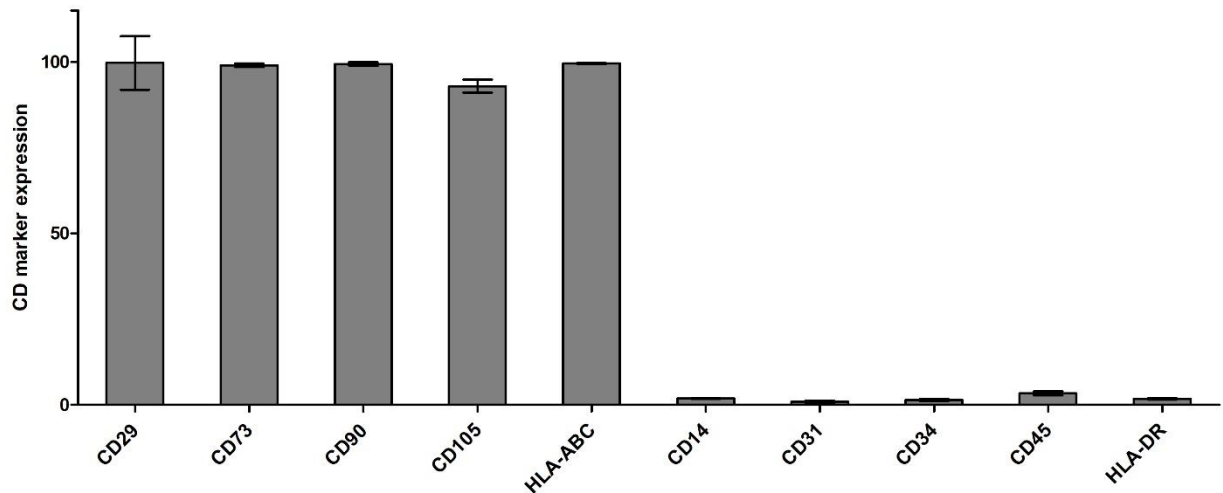
**Supplementary Table 1:** Population doubling time (PDT) in hours, and population doubling (PD) of WJ-MSCs at different passages

hWJ-MSC PD										
PassageNb.	1P	2P	3P	4P	5P	6P	7P	8P	9P	10P
PDT (days)	1.23 $\pm 0.15$	1.14 $\pm 0.14$	1.06 $\pm 0.14$	1.01 $\pm 0.13$	1.40 $\pm 0.19$	1.68 $\pm 0.17$	1.61 $\pm 0.15$	1.75 $\pm 0.18$	1.84 $\pm 0.04$	2.08 $\pm 0.04$
PD (index)	3.37 $\pm 0.31$	3.99 $\pm 0.40$	3.71 $\pm 0.22$	4.13 $\pm 0.23$	4.13 $\pm 0.32$	4.23 $\pm 0.30$	3.50 $\pm 0.37$	3.81 $\pm 0.29$	3.54 $\pm 0.04$	3.63 $\pm 0.02$



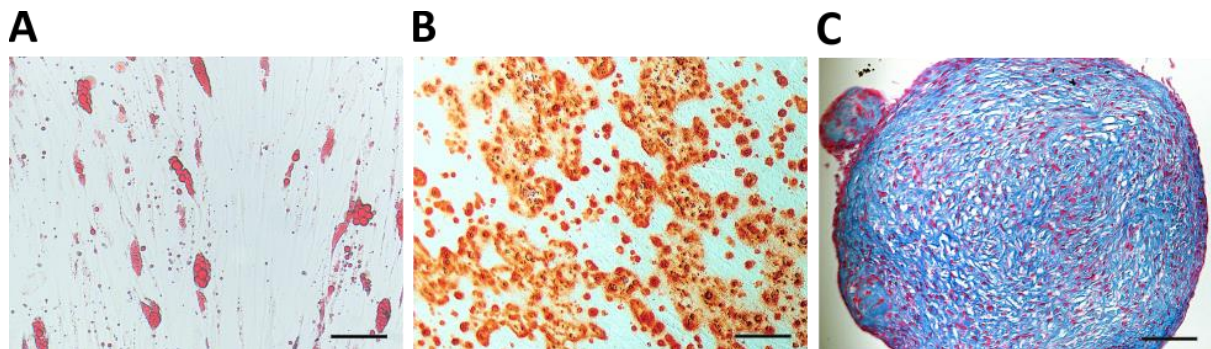
*Fig 1. cumulative population doubling (cPD) of hWJ-MSCs in different passages. Data are shown as mean  $\pm$  standard error mean,  $n = 7$ .*

The mesenchymal phenotype of hWJ-MSCs was confirmed by the presence of CD29, CD73, CD90, CD105 and HLA-ABC and lack of CD14, CD31, CD34, CD45 and HLA-DR (Fig. 2).



*Fig. 2. Flow cytometry analysis of the surface markers of hWJ-MSCs in the 3<sup>rd</sup> passage. Data are shown as mean  $\pm$  standard error mean,  $n = 7$ .*

hWJ-MSCs at the 3<sup>rd</sup> passage were differentiated into all three cell-lineages: adipocytes, osteoblasts, chondrocytes (Fig 3). Adipocytes were detected by positive Oil-Red-O staining for lipid droplets. Osteoblasts were detected by positive Alizarin Red S staining for calcium-rich deposits. Chondrocytes were detected by Alcian blue staining for acid mucopolysaccharides.



*Fig.3. Multipotent differentiation of human Wharton jelly mesenchymal stromal cells (hWJ-MSCs) Differentiation into (A) adipocytes, (B) osteoblasts, and (C) chondrocytes. The scale represents 100  $\mu$ m.*

1. Bruder, S.P., N. Jaiswal, and S.E. Haynesworth, *Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation*. J Cell Biochem, 1997. 64(2): p. 278-94.
2. Greenwood, S.K., et al., *Population doubling: a simple and more accurate estimation of cell growth suppression in the in vitro assay for chromosomal aberrations that reduces irrelevant positive results*. Environ Mol Mutagen, 2004. 43(1): p. 36-44.
3. Koci, Z., et al., *Characterization of human adipose tissue-derived stromal cells isolated from diabetic patient's distal limbs with critical ischemia*. Cell Biochem Funct, 2014. 32(7): p. 597-604.
54. Koci, Z., et al., *Extracellular Matrix Hydrogel Derived from Human Umbilical Cord as a Scaffold for Neural Tissue Repair and Its Comparison with Extracellular Matrix from Porcine Tissues*. Tissue Eng Part C Methods, 2017. 23(6): p. 333-345.