membranes

MDPI

Supplementary Material

Human Mesenchymal Stem Cells Seeded on the Natural Membrane to Neurospheres for Cholinergic-Like Neurons

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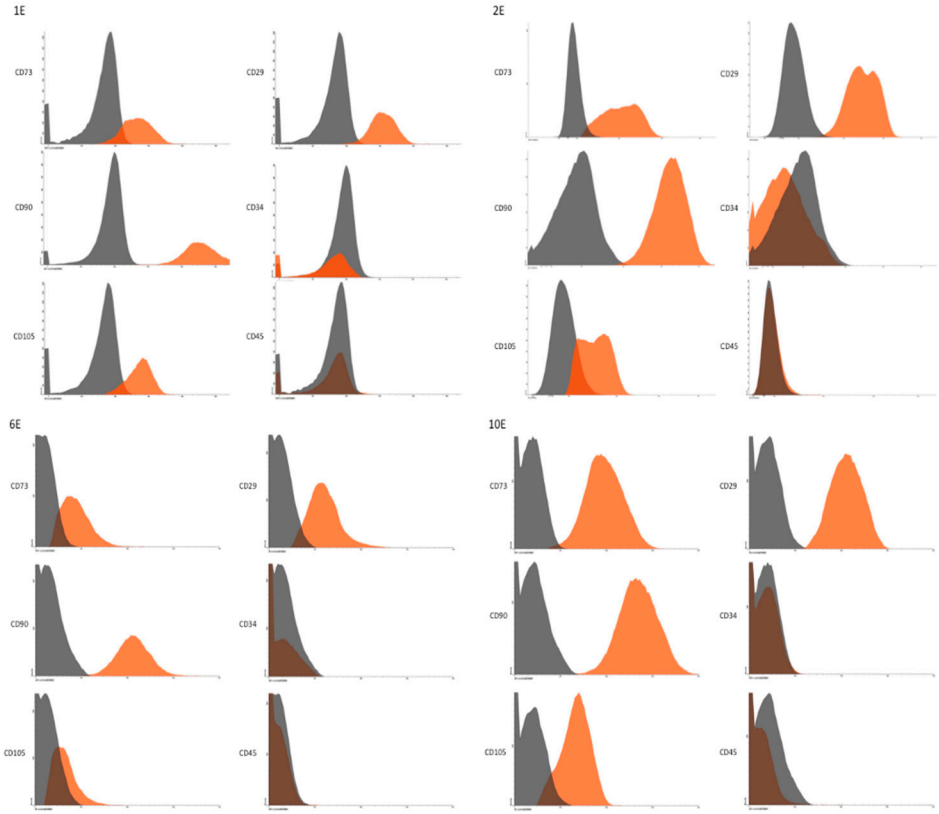
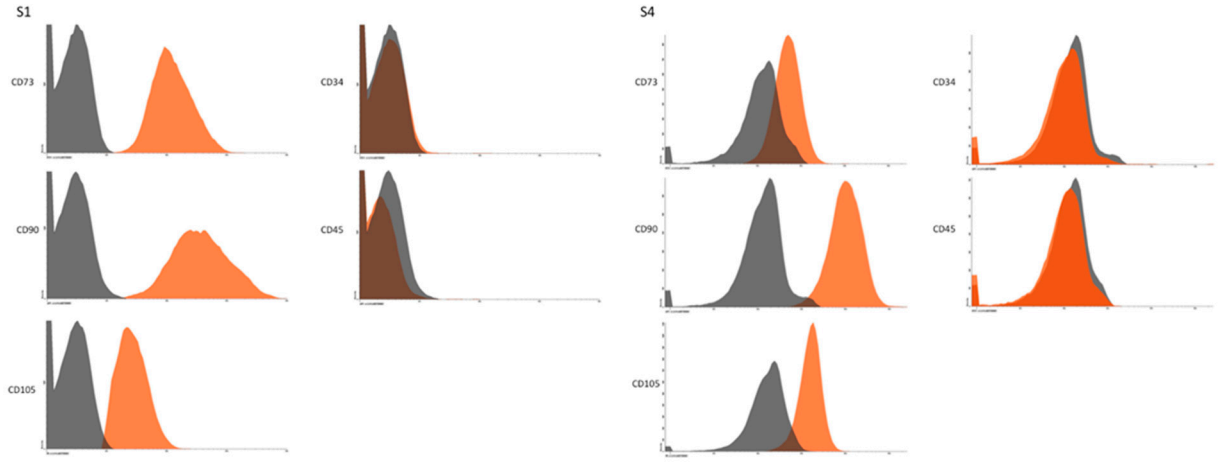


Surface marker expression of MSC-WJ. Note: SD – standard deviation. Referring to samples 1E, 2E, 6E and 10E.

Marker	CD73/CD105/CD90 (+)	CD73/CD29 (+)	CD34/CD45 (-)	7AAD (+)
1E	87.72	94.28	99.90	7.45
2E	91.75	89.61	99.97	15.67
6E	91.83	–	99.67	34.54
10E	93.76	–	99.99	12.95
Mean	91.27%	91.95%	99.88%	17.65%
SD	0.03	0.03	0.00	0.12



Surface markers expression of MSC-- - -UCB. Note: SD – standard deviation. Referring to samples S1 and S4.

Marker	CD73/CD105/CD90 (+)	CD34/CD45 (-)	7AAD (+)
S1	90.26	99.75	11.47
S4	92.33	99.95	6.49
Mean	91.30%	99.85%	10.53%
SD	1.46	0.13	6.01

Figure S1. Flow Cytometry Histograms and table analysis. (A) Histograms for the samples 1E, 2E, 6E, and 10E from MSC-WJ, and the respective table showing the statistic values. (B) Histograms for the samples S1 and S4 from MSC-UCB, and the respective table showing the statistic values. The gray peaks represent the isotype controls determining the area in which the marker is negative. The orange peaks correspond to the sample marking with its respective immunostaining marker, which is positive when the right of the gray peak and negative when on top or the left of the gray peak. Flow Cytometer was performed in FACS Calibur; Becton Dickinson, USA, and the analysis were conducted using Infinicyt Flow Cytometry Version 1.6.0 software.

Table S1. Cholinergic differentiation protocol in accordance Adib et al.,2015. A different concentration of growth factors and B27 per mL of medium was required throughout the 11 days of the protocol.

Day	NGF ng/mL	EGF ng/mL	bFGF ng/mL	B27 %
0	0	10	10	1
1	10	9	9	0.9
2	20	8	8	0.8
3	30	7	7	0.7
4	40	6	6	0.6
5	50	5	5	0.5
6	60	4	4	0.4
7	70	3	3	0.3
8	80	2	2	0.2
9	90	1	1	0.1
10	100	0	0	0