Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Effect of extracellular matrix proteins on the differentiation of human pluripotent stem cells into mesenchymal stem cells

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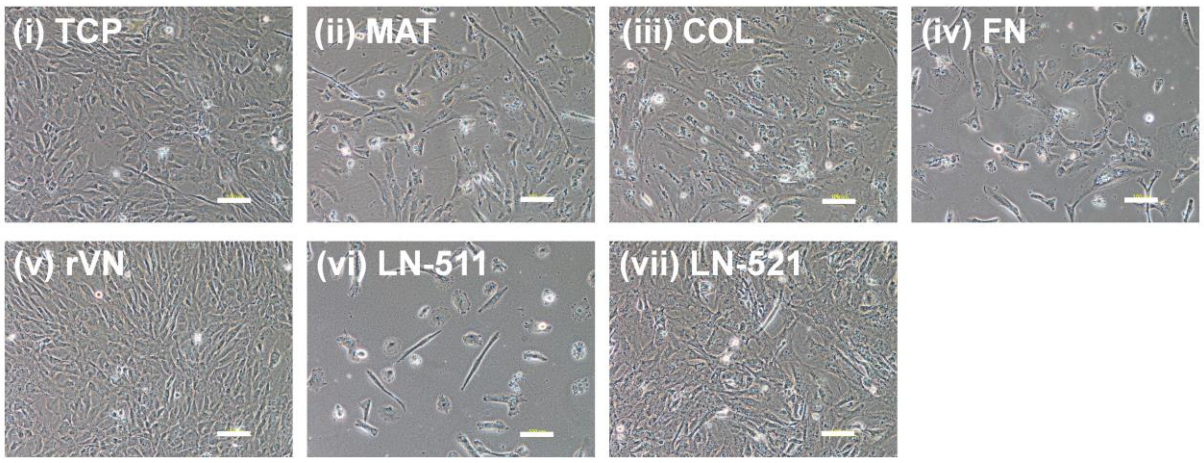
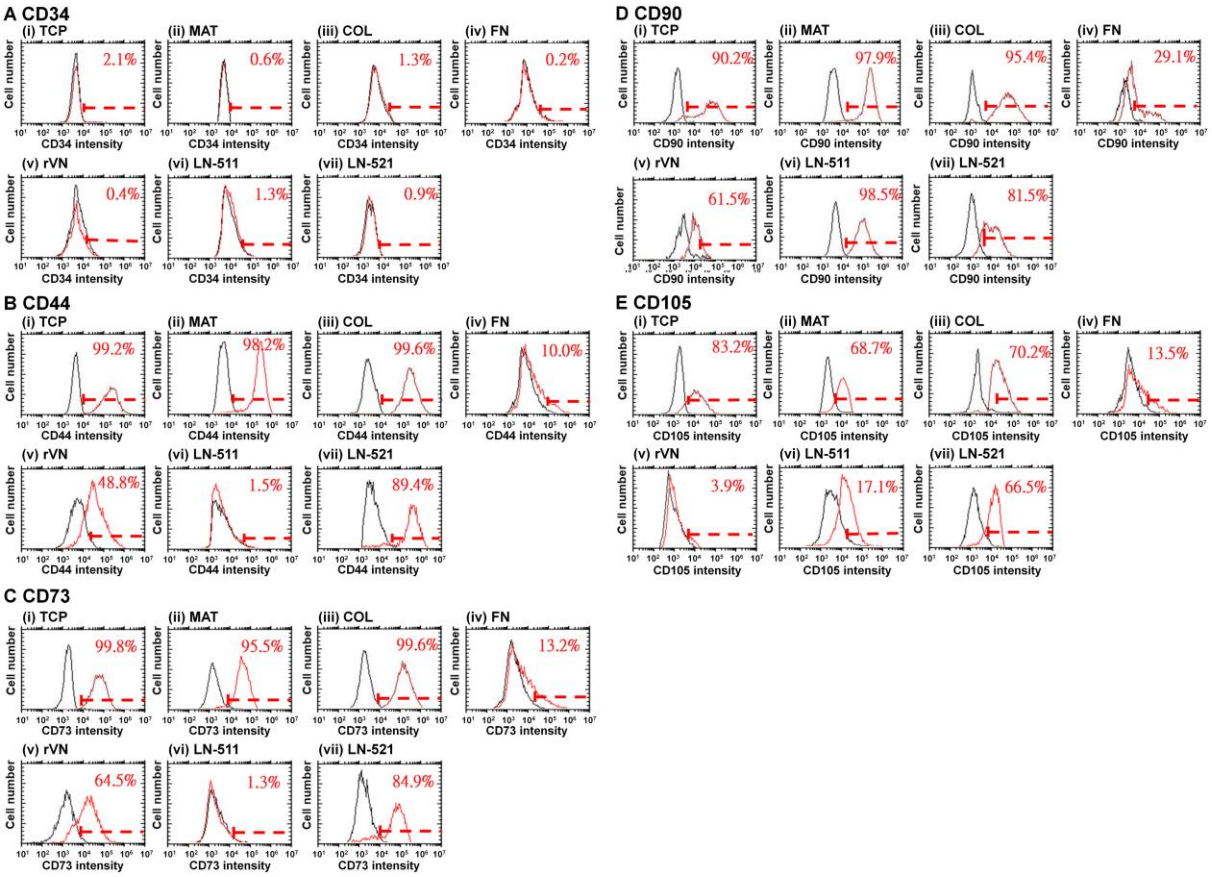
Supplementary Information

Supplementary Table 1 Materials used in this study.

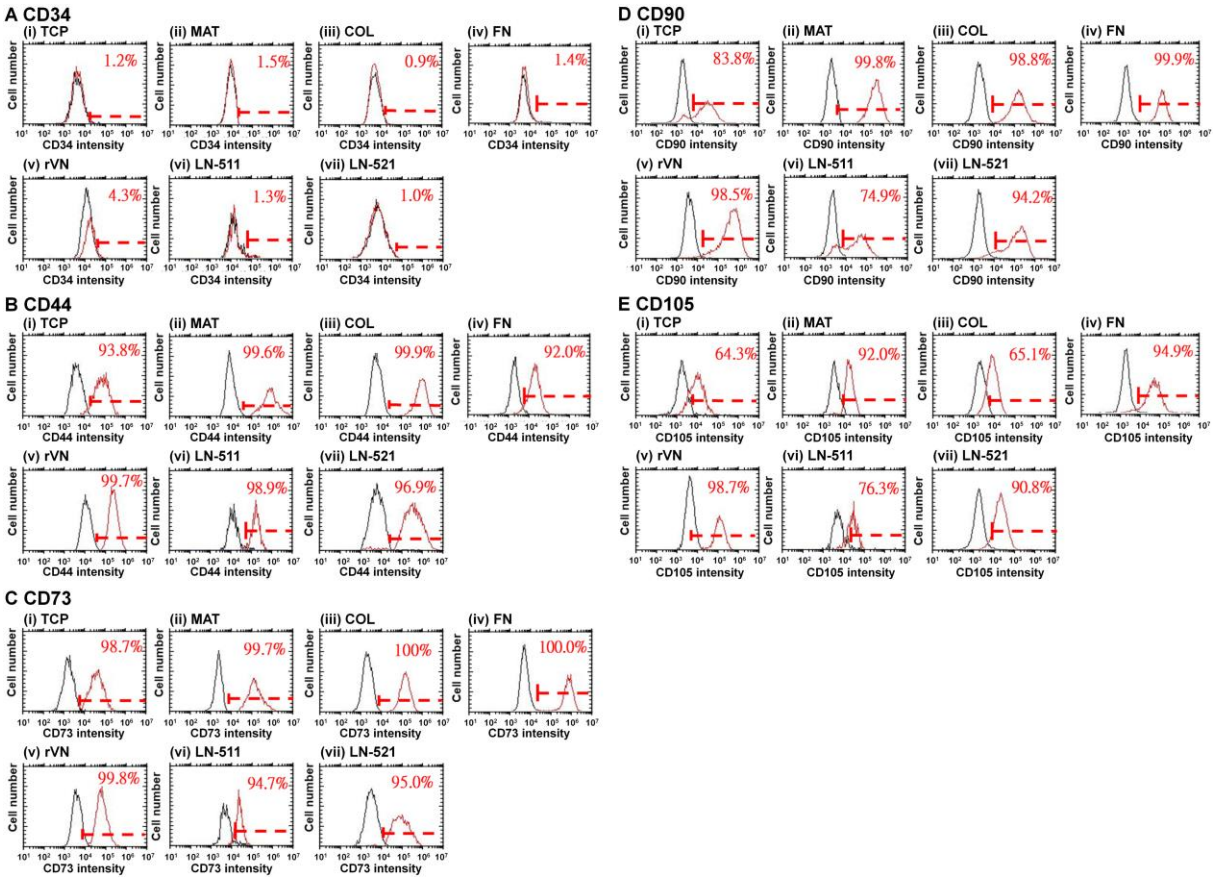
Materials	Abbreviation	Catalog No.	Company
		ECM	
Matrigel	MAT	356230	Corning (Corning, NY, USA)
Recombinant vitronectin	rVN	A14700	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Collagen type I	COL	354231	Corning (Corning, NY, USA)
Fibronectin	FN	33016-015	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Laminin 511	LN-511	892011	Nippi Inc. (Tokyo, Japan)
Laminin 521	LN-521	BLA-LN521-05	Veritas (Tokyo, Japan)
		Cell culture dishes	
6-well polystyrene plate	TCP	#353046	Corning (Corning, NY, USA)
		Chemicals	
Dispase II	Dispase	D4693-1G	Sigma-Aldrich (St. Louis, MO, USA)
Trypsin-EDTA (0.25%)	Trypsin-EDTA	25200072	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Bone morphogenic protein 4	BMP4	H4916	Sigma-Aldrich (St. Louis, MO, USA)
3-(6-Methyl-2-pyridinyl)-N-phenyl-4-(4-quinolinyl)-1H-pyrazole-1-carbothioamide	A83-01	SML0788	Sigma-Aldrich (St. Louis, MO, USA)
L-ascorbic acid-2-phosphate	L-ascorbic acid-2-phosphate	A8960-5G	Sigma-Aldrich (St. Louis, MO, USA)
Dexamethasone	DEX	D4902-500G	Sigma-Aldrich (St. Louis, MO, USA)
		Cell culture medium and component	
Essential 8 medium	Essential 8	A1517001	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Essential 6 medium	Essential 6	A1516401	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM/F12 medium	DMEM/F12	11330-057	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
α-MEM (Minimum Essential Medium)	α-MEM	12000022	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM	DMEM	D5648	Sigma-Aldrich (St. Louis, MO, USA)
Fetal bovine serum	FBS	PSRPS-FB2	Biological Industries (Kibbutz Beit-Haemek, Israel)
Alkaline Phosphatase Assay Kit	SensoLyte® pNPP	AS-72146	AnaSpec, Inc. (Fremont, CA, USA)
		Antibodies	
FITC Mouse Anti-Human CD34	Anti-CD34 antibody	555821	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse IgG1	Anti-CD34 isotype antibody	555748	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse Anti-Human CD44	Anti-CD44 antibody	555478	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse IgG2b	Anti-CD44 isotype antibody	555742	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse Anti-Human CD73	Anti-α-actinin antibody	550257	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse Anti-Human CD90	Anti-MLC2v antibody	555596	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse IgG1	Anti-CD73 (CD90) isotype antibody	555749	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse Anti-Human CD105	Anti-CD105 antibody	A07414	Beckman Coulter (Brea, CA, USA)
PE Mouse IgG3 RPE	Anti-CD105 isotype antibody	0105-09	SouthernBiotech (Birmingham, AL, USA)



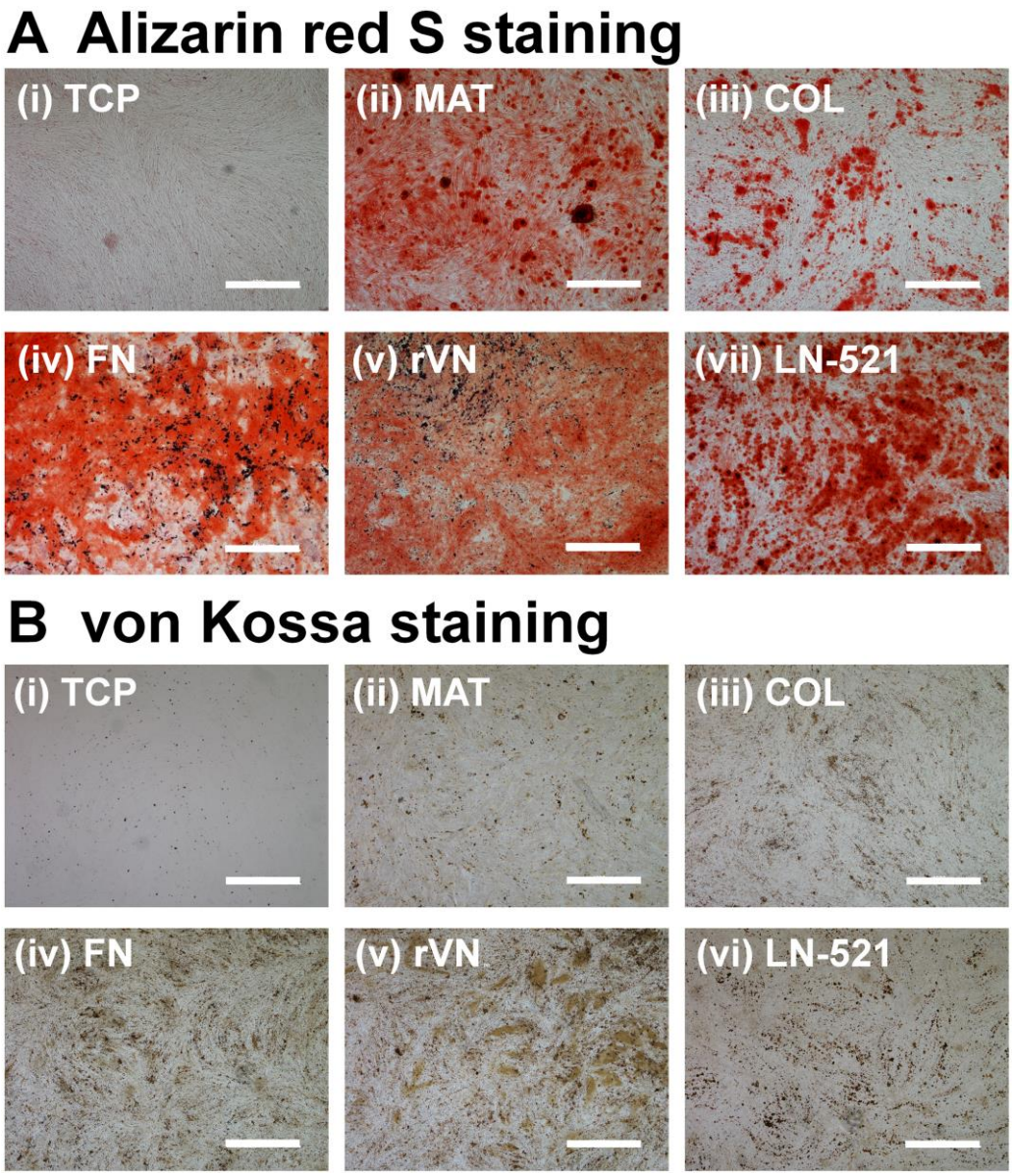
Supplementary Fig. 1. Differentiation of hiPSCs (H-M5) into hMSCs. Morphology of differentiated cells cultured on TCP dishes (i), MAT-coated dishes (ii), COL-coated TCP dishes (iii), FN-coated TCP dishes (iv), rVN-coated TCP dishes (v), LN-511-coated TCP dishes (vi) and LN-521-coated TCP dishes (vii) on day 7 at passage 10. The scale bar indicates 100 μm.



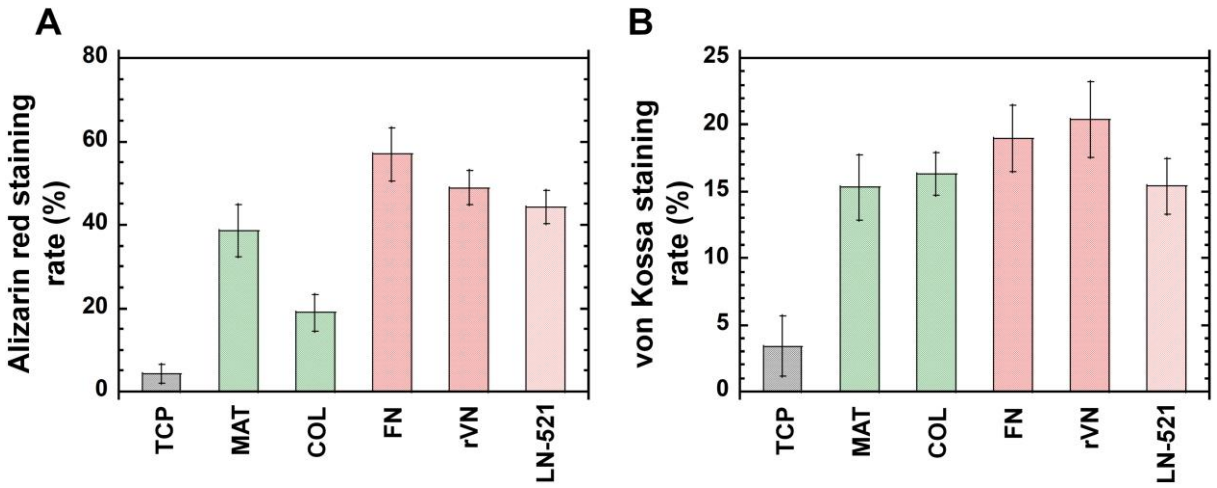
Supplementary Fig. 2. Flow cytometry histograms showing the expression of hMSC surface markers (CD44, CD70, CD93 and CD105) and a hematopoietic stem cell marker (CD34) on hESC (H9)-derived hMSCs cultured on TCP dishes and ECM-coated dishes at passage 6. (A-E) CD34 (A), CD44 (B), CD73(C), CD90 (D) and CD105 (E) expression on cells cultured on TCP dishes (i), MAT-coated dishes (ii), COL-coated TCP dishes (iii), FN-coated TCP dishes (iv), rVN-coated TCP dishes (v), LN-511-coated TCP dishes (vi) and LN-521-coated TCP dishes (vii). The black line illustrated the isotype antibody expression on the cells (negative control). The red line illustrated the expression of each CD marker on the cells.



Supplementary Fig. 3. Flow cytometry histograms showing the expression of hMSC surface markers (CD44, CD70, CD93 and CD105) and a hematopoietic stem cell marker (CD34) on hiPSC (H-M5)-derived hMSCs cultured on TCP dishes and ECM-coated dishes at passage 6. (A-E) CD34 (A), CD44 (B), CD73(C), CD90 (D) and CD105 (E) expression on cells cultured on TCP dishes (i), MAT-coated dishes (ii), COL-coated TCP dishes (iii), FN-coated TCP dishes (iv), rVN-coated TCP dishes (v), LN-511-coated TCP dishes (vi) and LN-521-coated TCP dishes (vii). The black line illustrated the isotype antibody expression on the cells (negative control). The red line illustrated the expression of each CD marker on the cells.



Supplementary Fig. 4. Osteogenic differentiation of hiPSC (H-M5)-derived hMSCs on day 28 of differentiation. (A) Micrograph of alizarin red S (calcium deposition)-stained cells cultured on TCP dishes (i), MAT-coated dishes (ii), COL-coated TCP dishes (iii), FN-coated TCP dishes (iv), rVN-coated TCP dishes (v), LN-511-coated TCP dishes (vi) and LN-521-coated TCP dishes (vii). The bar indicates 1000 μm. (B) Micrograph of von Kossa (calcium phosphate deposition)-stained cells cultured on TCP dishes (i), MAT-coated dishes (ii), COL-coated TCP dishes (iii), FN-coated TCP dishes (iv), rVN-coated TCP dishes (v), LN-511-coated TCP dishes (vi) and LN-521-coated TCP dishes (vii). The bar indicates 1000 μm.



Supplementary Fig. 5. Osteogenic differentiation of hiPSC (H-M5)-derived hMSCs. (A) The level of osteogenic induction of cells cultured on TCP dishes and ECM-coated dishes was assessed by alizarin red S staining (calcium deposition) on day 28 of differentiation utilizing ImageJ software. (B) The level of osteogenic induction of cells cultured on TCP dishes and ECM-coated dishes was evaluated by von Kossa staining (calcium phosphate deposition) on day 28 of differentiation utilizing ImageJ software.