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Culture and differentiation of purified human adipose-derived stem cells by membrane filtration via nylon mesh filters

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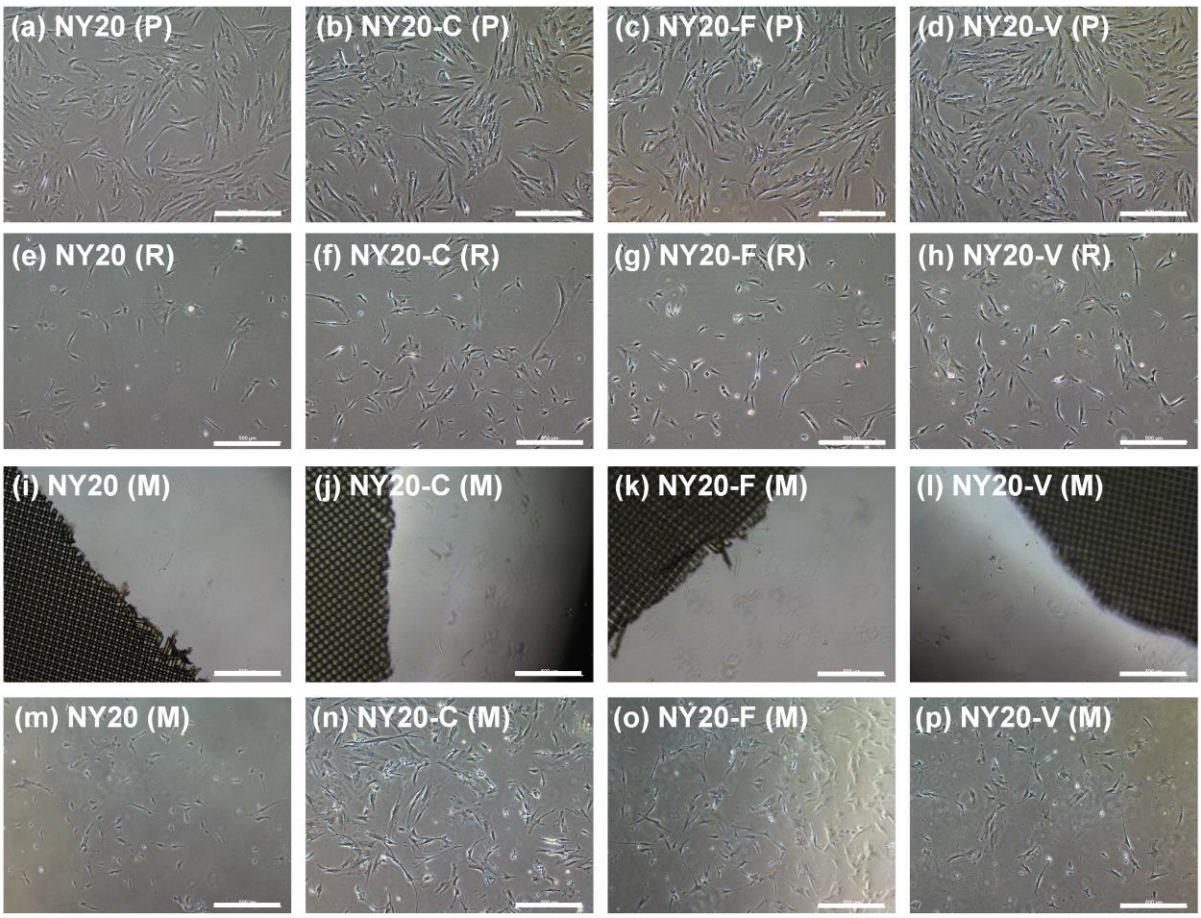
Table 1 Materials used in this study.

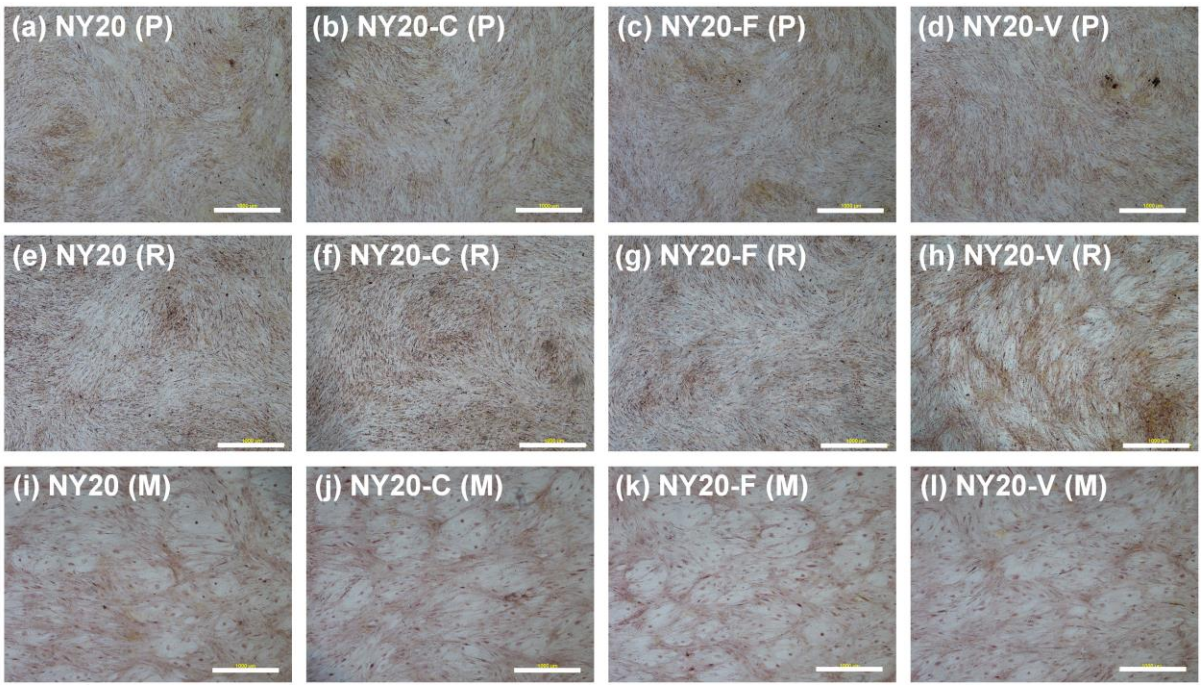
Materials	Abbreviation	Catalog No.	Company
	ECM		
Collagen Type I	COL	354231	BD Biosciences (San Jose, CA, USA)
Fibronectin, human	FN	#356008	Corning (Corning, NY, USA)
Recombinant vitronectin	rVN	A14700	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
	Cell culture dishes		
6-well tissue culture polystyrene plate	TCPS	#353046	Corning (Corning, NY, USA)
	Chemicals		
SensoLyte pNPP Alkaline Phosphatase Assay Kit	ALP kit	AS-72146	AnaSpec (Fremont, CA, USA)
	Cell culture medium and component		
DMEM	DMEM	D5648-10x1L	Sigma-Aldrich (St. Louis, MO, USA)
FBS	FBS	04-001-1A	Biological Industries, Kibbutz Beit-Haemek, Israel
Osteogenic differentiation medium	Mesenchymal Stem Cell Osteogenic differentiation medium	#7531	ScienCell Research Laboratories (Carlsbad, CA, USA)
Alizarin red S	Alizarin red S	A5533	Sigma-Aldrich (St. Louis, MO, USA)
Aqueous silver nitrate	Aqueous silver nitrate	S7276	Sigma-Aldrich (St. Louis, MO, USA)
Hoechst 33342	Hoechst	PA-3014	Lonza (Basel, Switzerland)
	Surface markers		
7-AAD Viability dye	7-AAD	559925	BD Biosciences (San Jose, CA, USA)
PE Mouse IgG1κ, isotype control	PE isotype	555749	BD Biosciences (San Jose, CA, USA)
FITC Mouse IgG1κ, isotype control	FITC isotype	555748	BD Biosciences (San Jose, CA, USA)
FITC Mouse IgG2bκ, isotype control	FITC isotype	555742	BD Biosciences (San Jose, CA, USA)
FITC mouse anti-human CD34	FITC anti-CD34	555821	BD Biosciences (San Jose, CA, USA)
FITC mouse anti-human CD44	FITC anti-CD44	555478	BD Biosciences (San Jose, CA, USA)
PE Mouse Anti-Human CD73	PE anti-CD73	550257	BD Biosciences (San Jose, CA, USA)
PE mouse anti-human CD90	PE anti-CD90	555596	BD Biosciences (San Jose, CA, USA)

Table 1 (continued)

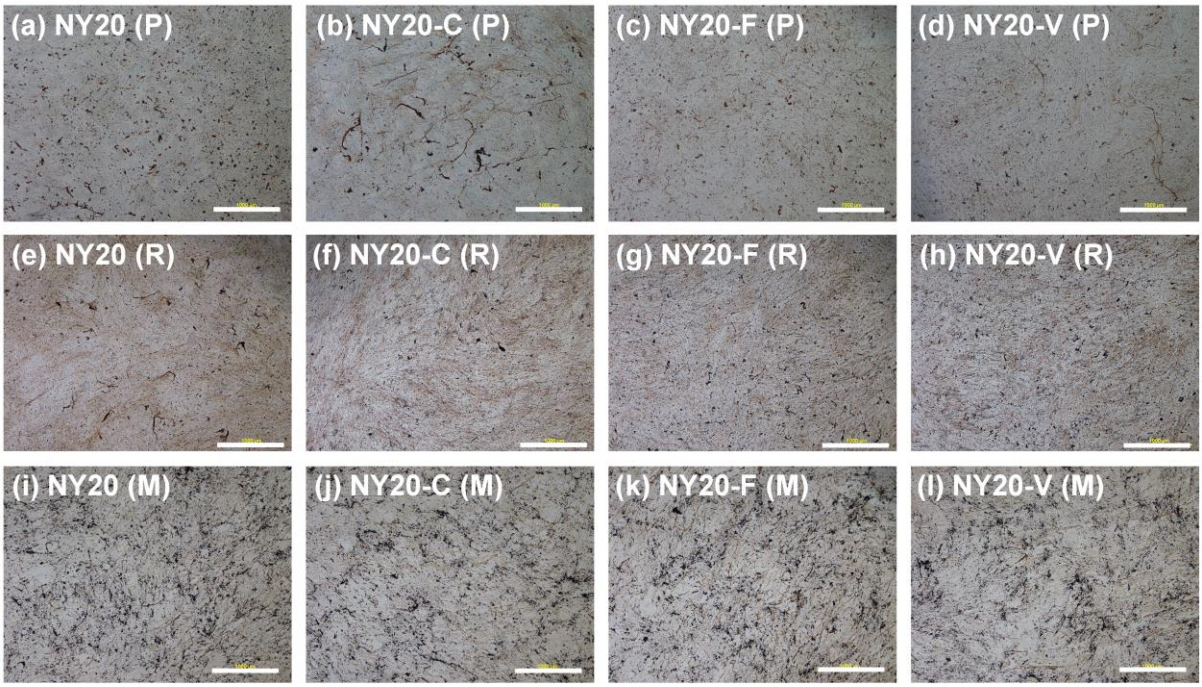
Materials	Abbreviation	Catalog No.	Company
	Filters		
Nylon mesh filter (r=11 um)	NY11	NY1104700	Merck KGaA (Darmstadt, German)
Nylon mesh filter (r=20 um)	NY20	NY2004700	Merck KGaA (Darmstadt, German)
	Gene expression component		
RNA extract kit	Total RNA Mini Kit	NR-S050	NovelGene Biotech Corporation (Taipei, Taiwan)
Reverse transcription	SuperScript First-Strand Reverse Transcriptase	11904-018	Thermo Fisher Scientific Inc. (Waltham, MA USA)
qPCR reagent	TaqMan Fast Universal PCR Master Mix	4352042	Thermo Fisher Scientific Inc. (Waltham, MA USA)
qPCR probe	GAPDH	Hs01047973_m1	Thermo Fisher Scientific Inc. (Waltham, MA USA)
qPCR probe	Runx2	Hs01866874_s1	Thermo Fisher Scientific Inc. (Waltham, MA USA)
qPCR probe	Osterix	Hs03929097_g1	Thermo Fisher Scientific Inc. (Waltham, MA USA)



Supplementary Fig. 1. Cell morphologies of hASCs purified by the membrane filtration method and membrane migration method. The cell morphologies of hASCs in the permeation solution (P) passed through the NY20 (a), NY20-C (b), NY20-F (c), and NY20-V (d) mesh filter membranes after 5 days of culture in TCP dishes. The cell morphologies of hASCs in the recovery solution (R) passed through the NY20 (e), NY20-C (f), NY20-F (g), and NY20-V (h) mesh filter membranes after 5 days of culture in TCP dishes. The cell morphologies of migrated cells (M) from the NY20 (i), NY20-C (j), NY20-F (k), and NY20-V (l) mesh filter membranes after 5 days of culture in TCP dishes. The cell morphologies of migrated cells (M) from the NY20 (m), NY20-C (n), NY20-F (o), and NY20-V (p) mesh filter membranes after 19 days of culture in TCP dishes. The bar indicates 500 μm.



Supplementary Fig. 2. Osteogenic differentiation of hASCs after filtration through NY20 filter membranes by the membrane filtration method and membrane migration method. (A) Micrograph of cells analyzed by Alizarin Red S staining (calcium deposition) on day 28 after osteogenic induction of (i) hASCs in permeation solution (P) (a-d), recovery solution (R) (e-h) and (ii) cells that migrated (M) (i-l) through NY20 mesh filter membranes (a, e, i) and NY20 mesh filter membranes coated with COL (b, f, j), FN (c, g, k) and rVN (d, h, l) after culture in TCP dishes for a week. The bar indicates 1000 μm.



Supplementary Fig. 3. Osteogenic differentiation of hASCs after filtration through NY20 filter membranes by the membrane filtration method and membrane migration method. Micrograph of cells evaluated by von Kossa staining (calcium phosphate deposition) on day 28 after osteogenic induction of (i) hASCs in permeation solution (P) (a-d), recovery solution (R) (e-h) and (ii) cells that migrated (M) (i-l) through NY20 mesh filter membranes (a, e, i) and NY20 mesh filter membranes coated with COL (b, f, j), FN (c, g, k), and rVN (d, h, l) after culture in TCP dishes for a week. The bar indicates 1000 μm.