Electronic Supplementary Material (ESI) for Biomaterials Science.

Substrate Stiffness Affects the Immunosuppressive and Trophic Function of hMSCs Via Modulating Cytoskeletal Polymerization and Tension

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A

	Acry%/Bis%	Elastic modulus(kPa)
S (soft)	6.5/0.01	0.5
M (medium)	4/0.2	50
R (rigid)	40/3	200

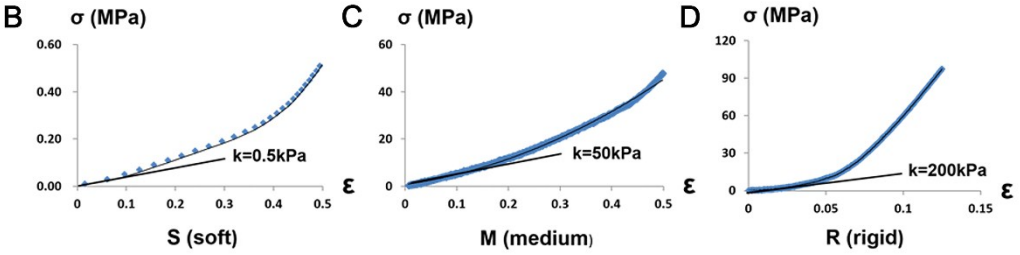

Fig. S1 Mechanical characterization of PAAm hydrogels with different crosslinking degrees. (A) Overview of PAAm hydrogel preparation. (B) Stress-strain curve for soft PAAm hydrogel (S). (C) Stress-strain curve for medium PAAm hydrogel (M). (D) Stress-strain curve for rigid PAAm hydrogel (R).

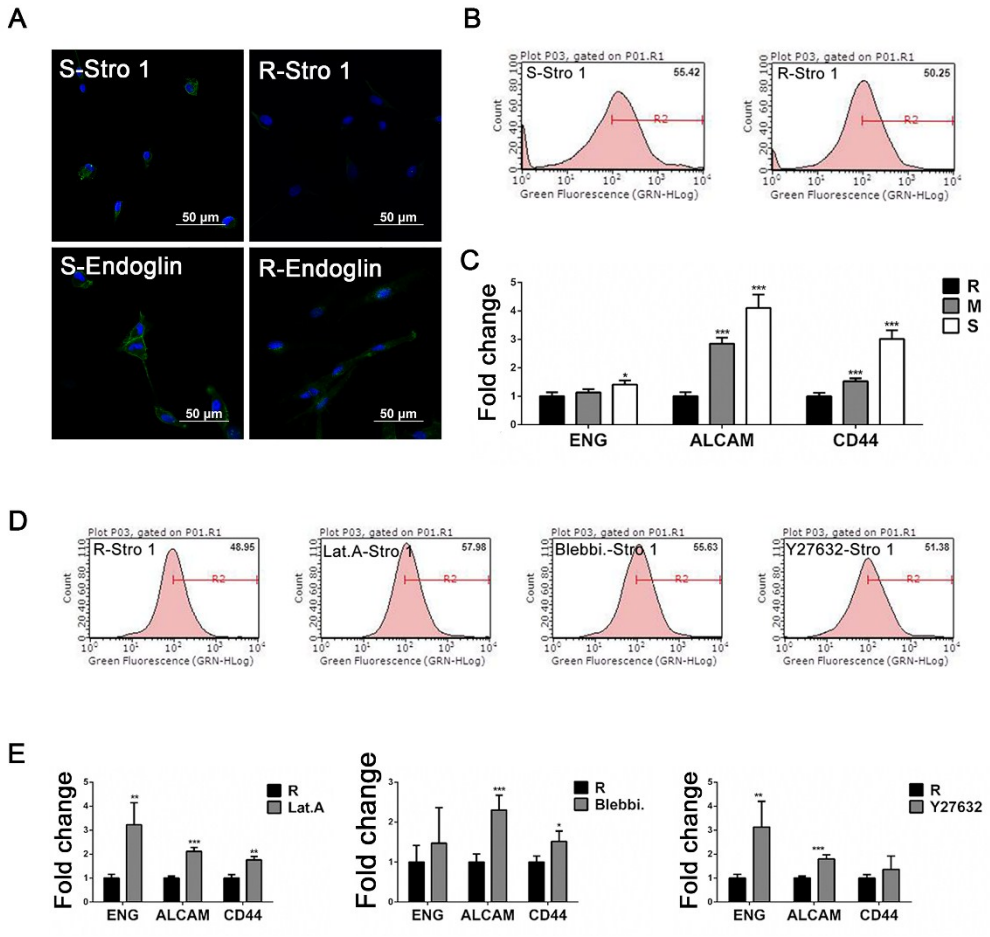

Fig. S2 Soft substrates and cytoskeletal inhibition helped 'stemness' retention of hMSCs. (A) Immunofluorescent staining of stro-1 and endoglin for hMSCs cultured on substrates with different stiffness. (B) Flow cytometry analysis of stro-1 for hMSCs cultured on substrates with different stiffnesses. (C) 'Stemness' related gene expression for hMSCs cultured on substrates with different stiffness. All the genes were normalized to GAPDH and 18 S rRNA. (D) Flow cytometry analysis of stro-1 after F-actin, myosin and ROCK inhibition. (E) 'Stemness' related gene expression after F-actin, myosin and ROCK inhibition. All the genes were normalized to GAPDH and 18 S rRNA.

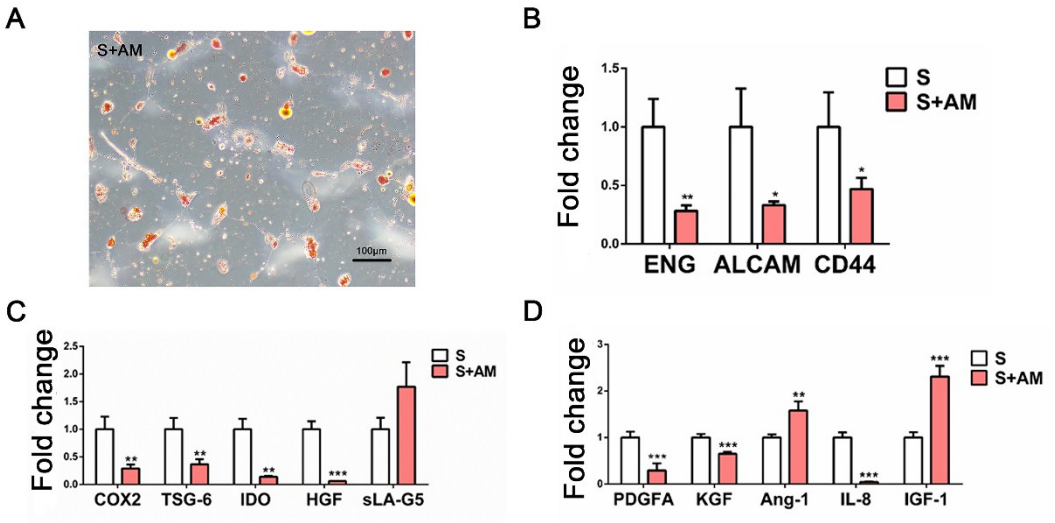

Fig. S3 The immunosuppressive and trophic capacities of hMSCs decreased after adipogenic differentiation. (A) Oil red O staining after 21 days of adipogenic induction for hMSCs cultured on soft substrates. (B) 'Stemness' related gene expression after 7 days of adipogenic induction for hMSCs cultured on substrates with different stiffness. All the genes were normalized to GAPDH and 18S rRNA. (C) Gene expression of immunomodulatory factors after 7 days of adipogenic induction. All the genes were normalized to GAPDH and 18S rRNA. (D) Gene expression of trophic factors after 7 days of adipogenic induction. All the genes were normalized to GAPDH and 18S rRNA.

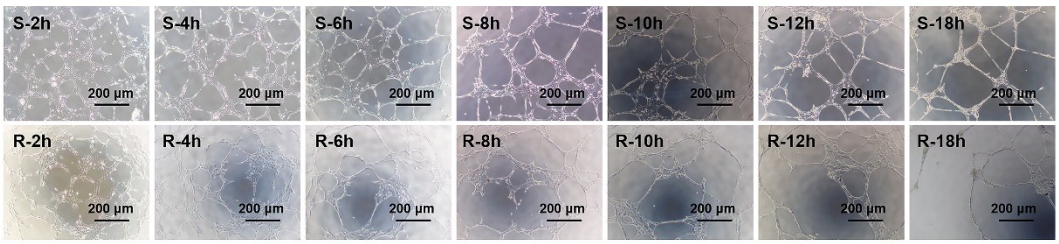

Fig. S4 Dynamic change during tube formation of HUVECs in different time points (from 2 hours to 18 hours).

Table S1. Primer sequences used for qRT-PCR human gene expression analysis

Gene	5'-3'	Primers
ENG	Sense	CAGACAAAGTGTGCCGACGA
	Anti-sense	TGGAGTAAGCACTGCGCAAGA
ALCAM	Sense	CATTATCATACCTTGCCGACTTG
	Anti-sense	TGTATTCTGGTACATCGTCGTACTG
CD44	Sense	GCATTGCAGTCAACAGTCGAAGA
	Anti-sense	CCTTGTTCACCAAATGCACCA
COX2	Sense	CAGAGCAGGCAGATGAAATACCAG
	Anti-sense	TTTCTACCAGAAGGGCAGGATACAG
TSG6	Sense	GTGGAGATGAGCTTCCAGATGAC
	Anti-sense	GGATACAGGATCCATTGCAACA
IDO	Sense	CTGGGAAGACCCAAAGGAGTT
	Anti-sense	CCTGGAGGAACTGAGCAGCA
HGF	Sense	GAATACTGCAGACCAATGTGCTAA
	Anti-sense	CACTCCACTTGACATGCTATTGA
sLA-G5	Sense	CCTTGCAGCTGTAGTCACTGGA
	Anti-sense	CACACAGGGCAGCTGTTTCA
PDGFA	Sense	CACTAAGCATGTGCCCGAGAA
	Anti-sense	CGTAAATGACCGTCCTGGTCTTG
KGF	Sense	CACAGTGGTACCTGAGGATCGATAA
	Anti-sense	AATTCCAACTGCCACTGTCCTG
Ang-1	Sense	GTGCAAATGTGCCCTCATGTTA
	Anti-sense	AGGTCGAATCATCATAGTTGTGGAA
IGF-1	Sense	TCTTCAGTTCGTGTGTGGAGACAG
	Anti-sense	GGGTGCGCAATACATCTCCAG
18S	Sense	ACTCAACACGGGAAACCTCA
	Anti-sense	AACCAGACAAATCGCTCCAC
GAPDH	Sense	GCACCGTCAAGGCTGAGAAC
	Anti-sense	TGGTGAAGACGCCAGTGGA

Table S2. Primer sequences used for qRT-PCR mouse gene expression analysis

Gene	5'-3'	Primers
IL-1β	Sense	TCCAGGATGAGGACATGAGCAC
	Anti-sense	GAACGTCACACACCAGCAGGTTA
TNFα	Sense	ACTCCAGGCGGTGCCTATGT
	Anti-sense	GTGAGGGTCTGGGCCATAGAA
NOS2	Sense	CAAGCTGAACTTGAGCGAGGA
	Anti-sense	TTTACTCAGTGCCAGAAGCTGGA
CD11c	Sense	TCATCATTCAAGCAGAGCCAGAAC
	Anti-sense	TACCCGAGCCATCAATCAGGA
ARG1	Sense	TGACCGCCGTCGTGTTACTTTA
	Anti-sense	TTCTCGCCCACTAGGCAGTTC
IL-1RA	Sense	TCTTGTGCCAAGTCTGGAGATGATA
	Anti-sense	AGCTGACTCAAAGCTGGTGGTG
IL-10	Sense	GCCAGAGCCACATGCTCCTA
	Anti-sense	GATAAGGCTTGGCAACCCAAGTAA
CD206	Sense	AGAGCTGGCGAGCATCAAGAG
	Anti-sense	TTCCATAGGTCAGTCCCAACCAA
GAPDH	Sense	AAATGGTGAAGGTCGGTGTGAAC
	Anti-sense	CAACAATCTCCACTTTGCCACTG