Investigation of Nanoscale Topography and Biomechanical Tuning of PDMS Substrates to Enhance Cardiomyocyte Differentiation from Human Induced Pluripotent Stem Cells

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Table S1. List of different substrates, which were used for iCM differentiation.

Substrates	Stiffness	Topography and Roughness	Representative
10:1 Plain	~667kPa (Hard)	• Non-topographic • Smooth (~3.6 nm)	Chemical+Mechanical (Duplicate Factors)
10:1 Cell-Imprinted	~667kPa (Hard)	• Human Cardiomyocyte Pattern • Rough (~112 nm)	Chemical+Mechanical+Physical (Triple factors)
30:1 Plain	~106kPa (Soft)	• Non-topographic • Smooth (~3.6 nm)	Chemical+Mechanical (Duplicate Factors)
30:1 Cell-Imprinted	~106kPa (Soft)	• Human Cardiomyocyte Pattern • Rough (~112 nm)	Chemical+Mechanical+ Physical (Triple Factors)
TCPS (Control Condition)	3GPa[35]	• No Pattern • ~2.5 nm[51]	Chemical (Single Factor)

Table S2. qPCR primer sequences for human gene markers: forward (F) and reverse (R).

GENE	Accession Number	Primer sequence (both 5'-3')	Annealing temperature (°C)	Product size (bp)
NANOG-F	NM_024865	ACATGCAACCTGAAGACGTGTG	61.63	144
NANOG-R		CATGGAAACCAGAACACGTGG	59.73	
T-R	NM_003181	CAACCTCACTGACGGTGAAAAA	59.32	101

T-F		ACAAATTCTGGTGTGCCAAAGTT	59.80	
NKX2-5-F	NM_001166176	ACCCTGAGTCCCCTGGATTT	60.18	125
NKX2-5-R		TCACTCATTGCACGCTGCAT	60.96	
HAND1-F	NM_004821	TCCCTTTTCCGCTTGCTCTC	60.32	114
HAND1-R		CATCGCCTACCTGATGGACG	60.32	
TNNT2-F	NM_001001430	TTCACCAAAGATCTGCTCCTCGCT	64.18	166
TNNT2-R		TTATTACTGGTGTGGAGTGGGTGTGG	64.34	
MYH7-F	NM_000257	ACCAACCTGTCCAAGTTCCG	55.00	128
MYH7-R		TTCAAGCCCTTCGTGCCAAT	50.00	
TNNI3-F	NM_000363	CCAACTACCGCGCTTATGC	59.36	120
TNNI3-R		CTCGCTCCAGCTCTTGCTTT	60.67	
GAPDH-F	NM_001357943	AATCCCATCACCATCTTCCAG	57.41	122
GAPDH-R		AAATGAGCCCCAGCCTTC	57.23	

Table S3. List of primary and secondary antibodies.

Cell	**Protein**	**Primary Antibody**	**Secondary Antibody**	**conditions**
hiPSC(253G1)	NANOG	Rabbit polyclonal IgG (H-155), sc-33759, Santa Cruz Biotechnology.	Goat anti-rabbit IgG, Alexa Fluor 488, A-11008, Invitrogen.	Primary: room temperature, one hour Secondary: darkness, 30 min
	OCT-3/4	Mouse monoclonal IgG2b (C-10), sc-5279, Santa Cruz Biotechnology.	Goat anti-mouse IgG2b, Alexa Fluor 594, A-21145, Invitrogen.	
Cardiomyocyte	cTnT	Mouse monoclonal IgG2a, MAB 1874, R&D.	Goat anti-mouse IgG2a, Alexa Fluor 594, A-21135, Invitrogen	Primary: room temperature, one hour Secondary: darkness,

	MHY7	Rabbit Monoclonal Antibody IgG, MAB 90961, R&D.	Goat anti-rabbit IgG, Alexa Fluor 488, A-11008, Invitrogen.	30 min
Human Fetus Cardiomyocytes (HFCMs)	α-actinin	Anti-Sarcomeric Alpha Actinin antibody (ab137346) (ab137346, abcam, USA)	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077, abcam, USA).	Primary: room temperature, one hour Secondary: darkness, 30 min