Dual role of electrical stimulation and biomimetic matrix on neural differentiation within the microfluidic platform

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Supplementary Figures

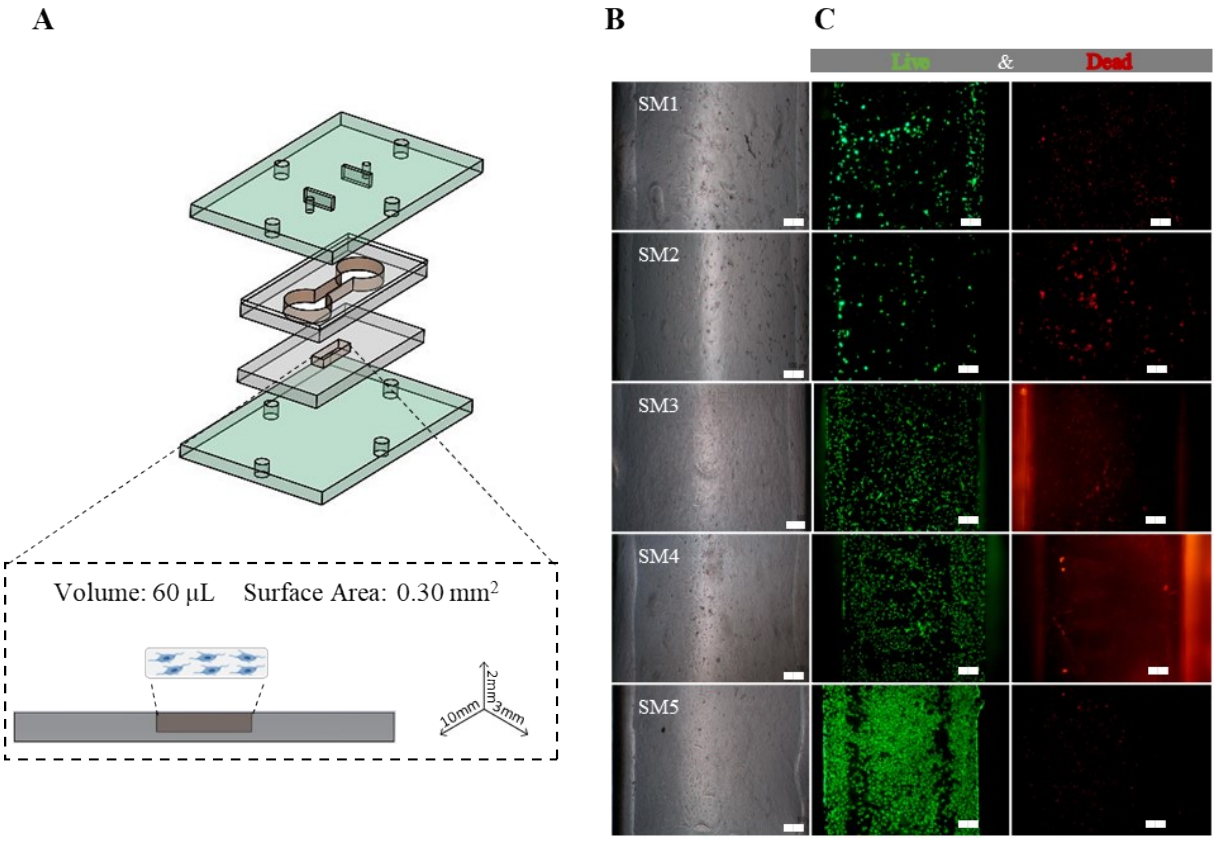

Fig. S1 Optimization of surface modification on µ-platform. (A) Design and dimensions of the µ-platform. The image was created with Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. Cellular viability efficiency on different surface modification procedures on day 1 with (B) light microscopy and (C) Live&Dead staining (Scale bars 100 µm). Cellular viability efficiency on different surface modification procedures on day 1 with (B) light microscopy and (C) Live&Dead staining (Scale bars 100 µm)"

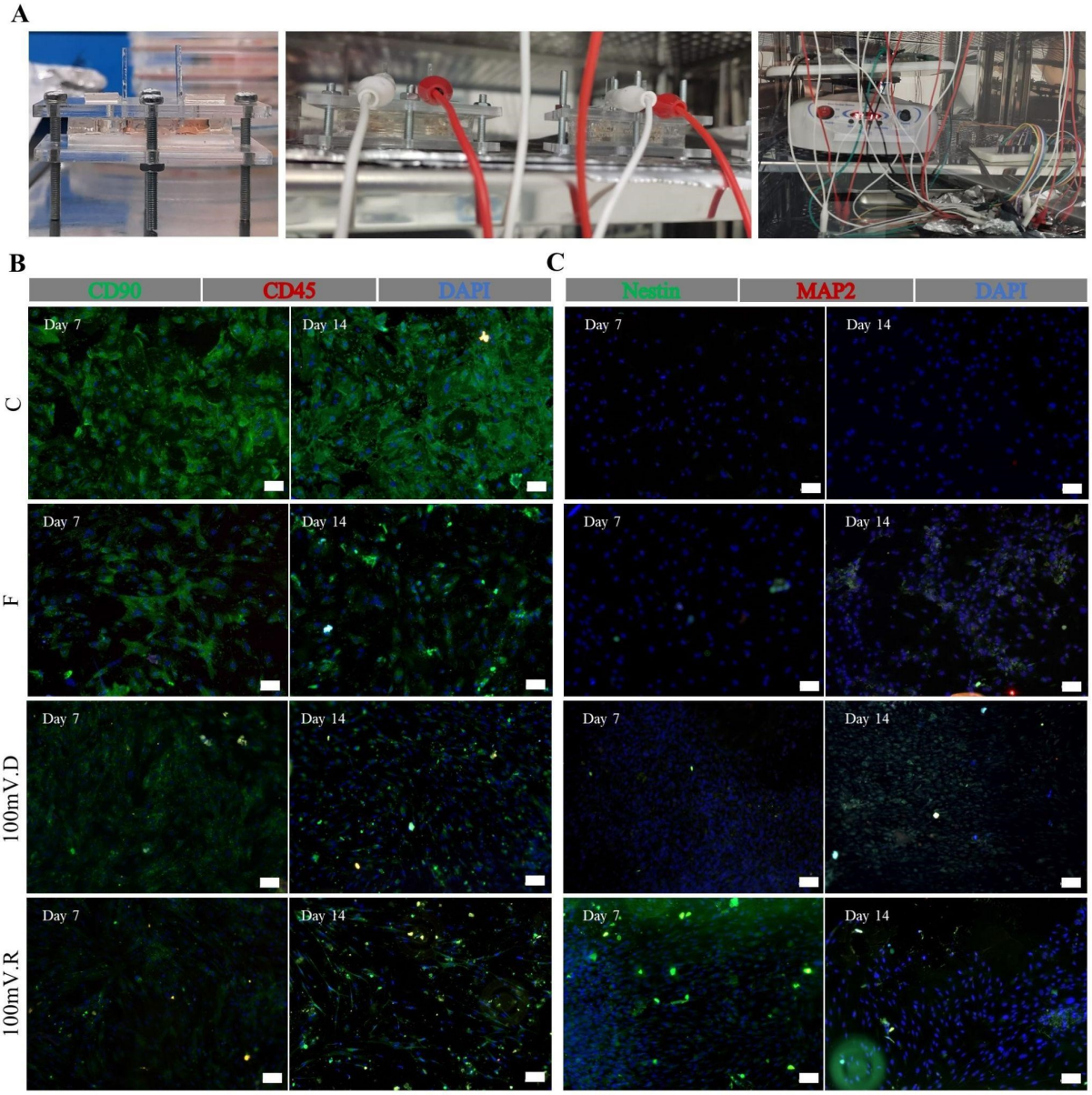

Fig. S2 The effect of electrical stimulation on neural differentiation of BMSC at 7 and 14 days. (A) Side view of the μ-platform, integration of electrical stimulation and followed by the generation of dynamic flow within the μ-platform. Immunofluorescence staining of (B) mesenchymal stromal cell markers (CD45 and CD90), (C) neural stem cells progenitor and mature phase cell markers (Nestin and MAP2) (Scale bars 100 μm).

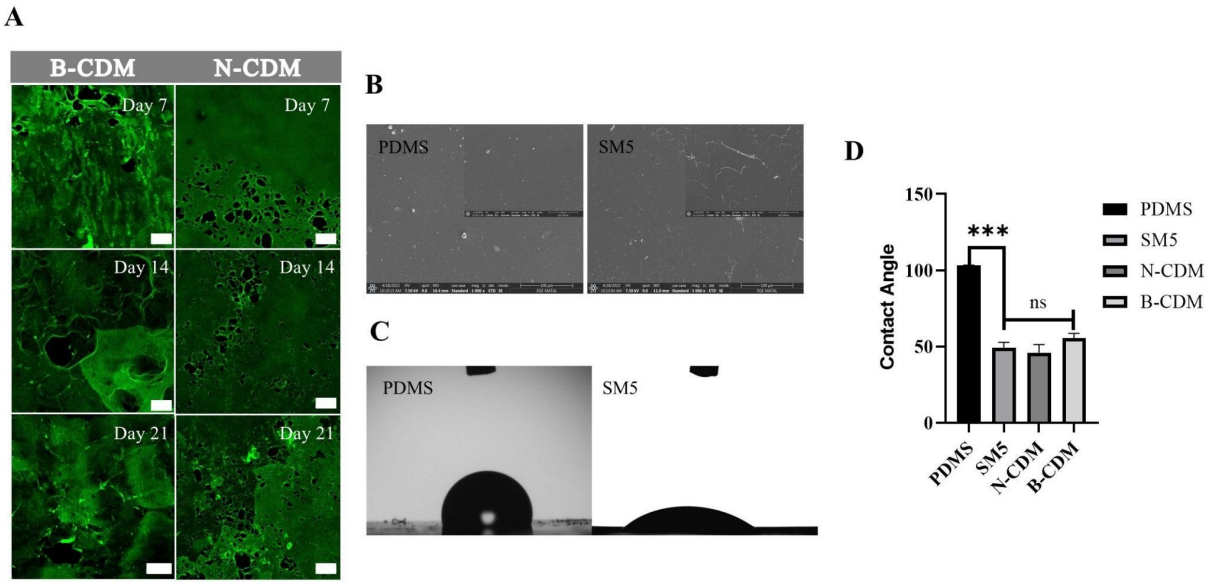

Fig. S3 Optimization of CDM production. (A) Immunofluorescence staining of CDMs with fibronectin (Scale bars 50 μm). (B) Surface structure analysis with SEM imaging. (C) Hydrophilicity analysis of PDMS and SM5 coated PDMS. (D) Contact angle measurements of PDMS, SM5 and CDMs for cell adhesion.

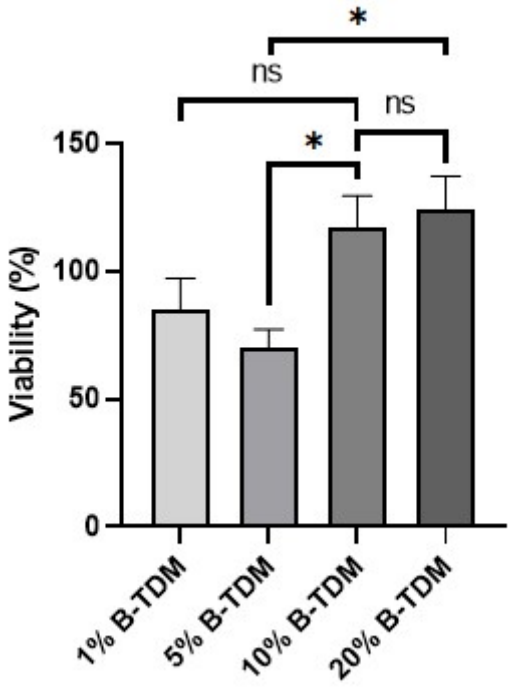

Fig. S4 Biocompatibility of B-TDM hydrogel. Viability of BMSCs embedded in different B-TDM pre-gel concentrations on day 1 (ns:non-significant $p>0.05$, *$p<0.05$).

Supplementary Tables

Table S1. *Antibody list for immunofluorescence dyes*

Antibody	Host	Supplier/cat. no	IF dilution	Region	Cellular localization
CD45	Rabbit	Abcam, ab10558	1:1000	Hematopoietic myeloid cells	Nucleus
CD90	Mouse	SantaCruz sc53116	1:500	Cluster of differentiation	Cytoplasm
Nestin	Mouse	SantaCruz, sc23927	1:500	CNC progenitor cells	Intermediate filament
MAP2	Chk	Abcam, ab5392	1:1000	Mature neurons	Cytoplasm, cytoskeleton
Fibronectin	Mouse	Biosciences, 610077BD	1:500	Extracellular matrix	Cytoskeleton organization

Table S2*. Primer list for qRT-PCR analysis*

Gene	5' forward 3'	5' reverse 3'
SOX2	TTGCTGCCTCTTTAAGACTAGGA	TAAGCCTGGGGCTCAAACT
TUJ1 (TUBB3)	GACCGGACGGTGAGTCAG	CACGAGGGAAGAAGTTTTGC
CDH2 (Ncad)	CTCCATGTGCCGGATAGC	CGATTTCACCAGAAGCCTCTAC
SOX1	TCTTTTGGGTTGGTTTGTTAATTT	AATATAACTCCGCCGTCTGAAG
PSD95	GACGGCCTACTTTACTCACAGC	CTCCAGGATTGGAGTTGAGC
TBR1	TAGCAGCGCTAACATCAGCA	CCGAGCCCTAAGCCTTAAA
CTIP2	GCTTTCCACCTACCAGACCC	ATCACGGATGAGTGAGGGTG
MAP2	ATGACCCCCTCATCCAAAG	CATGTGGCCAGACTCAACAC
NESTIN	ACCTGTGCCAGCCTTTCTTA	GCCAAGGTAGGGGTACGG
NEUN (RBFOX3)	CCCAACAGAAAGGGCTGAC	CTGGGCTTCCTTCGTCCT
IBA1	CTCCAGCTTGGAGGAAAAGC	TGGAGGGCAGATCCTCATCA
GFAP	CTTGCTTCAGCGGTCAGG	TGCATGAGGCAGGTGGTA
S100B	TGTAGACCCTAACCCGGAGG	TGCATGGATGAGGAACGCAT
OLIG2	TCGCATCCAGATTTTCGGGT	TCCATGGCGATGTTGAGGTC
MBP	CCCTGCACCTGTCAGTCC	GGCAGCTTTCTCACATACCG
CTNNB1	TGTTAAATTCTTGGCTATTACGACA	CCACCACTAGCCAGTATGATGA

Table S3. *Fold change and fold regulation data of BMSCs in 3D B-TDM in absence and presence of ES under static and dynamic conditions compared to BMSC control.*

Genes	**(Comparing to BMSC Control)**					
	BMSC Static ES-		**BMSC Static ES+**		**BMSC Dynamic ES+**	
	Fold Change	**Log2 Fold Regulation**	**Fold Change**	**Log2 Fold Regulation**	**Fold Change**	**Log2 Fold Regulation**
PAX6	4.63	2,21	370	8,53	14.49	3,86
CTIP2	0,003	-8,38	3.32	13,52	0.05	-4,32
SOX1	0.02	-5,64	0,03	-5,06	0.03	-5,06
SOX2	1.75	0,81	0,14	-2,84	1.82	0,86
NESTIN	0.04	-4,64	1.39	13,80	0.14	-2,84
NEUN	4.82	2,27	9.74	14,74	11.37	3,51
MAP2	0.01	-6,64	0,17	-2,56	0.45	-1,15
OLIG2	308.69	8,27	1392	10,44	536	9,07
MBP	0.26	-1,94	1.50	14,16	21.66	4,44
GFAP	4.99	2,32	1272	10,31	1495	10,55

S100B	6.15	2,62	18,29	4,19	8.26	3,05
IBA1	0,003	-8,38	4.67	14,58	0.07	-3,84

Table S4. *Fold change and fold regulation data of iPSCs in 3D B-TDM in the absence and presence of ES under dynamic conditions compared to wild-type iPSC.*

Genes	**(Comparing to iPSC Control)**			
	iPSC Dynamic ES-		**iPSC Dynamic ES+**	
	Fold Change	**Log2 Fold Regulation**	**Fold Change**	**Log2 Fold Regulation**
CDH2	0.01	-6,644	9.51	3,249
PAX6	0.25	-2,000	4.03	2,011
TBR1	0.12	-3,059	3.32	1,731
CTNNB1	0.13	-2,943	37.79	5,240
TUJ1	4.85	2,278	15.56	3,960
PSD95	3.55	1,828	3.32	1,731
CTIP2	8.32	3,057	183.55	7,520
SOX1	2.18	1,124	23.26	4,540
SOX2	29.58	4,887	44.63	5,480
Nestin	1.52	0,604	7.36	2,880

NEUN	0.20	-2,322	1.21	0,275
MAP2	0.28	-1,837	0.71	-0,494
Olig2	1.55	0,632	8.57	3,099
MBP	14.19	3,827	34.06	5,090
GFAP	0.75	-0,415	4.38	2,131
S100B	2.44	1,287	13.83	3,790
IBA1	0.78	-0,358	0.97	-0,044