Supporting Information

ELECTRICAL STIMULATION OF NEURAL-DIFFERENTIATING iPSCs ON NOVEL COAXIAL ELECTROCONDUCTIVE NANOFIBERS

Fábio F. F. Garrudo,[a,b,c,d,*] Diogo E. S. Nogueira,[b,c] Carlos A. V. Rodrigues,[b,c] Flávio A. Ferreira, [b,c] Patrizia Paradiso,[e] Rogério Colaço,[e] Ana C. Marques,[f] Joaquim M. S. Cabral,[b,c] Jorge Morgado,[d] Robert J. Linhardt,[a,*] Frederico Castelo Ferreira,[b,c,*]

[a] Department of Chemistry and Chemical Biology, Department of Chemistry & Chemical Biology, Rensselaer Polytechnic Institute, Biotechnology Center 4005, Troy, NY, 12180, USA.
[b] Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisboa, Portugal.
[c.] Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico,Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[d] Department of Bioengineering and Instituto de Telecomunicações, Universidade de Lisboa, Av. Rovisco Pais, P-1049-001, Lisboa, Portugal.
[e] IDMEC - Instituto de Engenharia Mecânica, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, P-1049-001 Lisboa, Portugal
[f] CERENA, DEQ, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais, P-1049-001 Lisboa, Portugal.

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Table S1: Composition of the solutions used in this work for fiber production.

Solution	PANI (mg)	CSA (mg)	PCL (g)	PGS (g)	TFE (mL)	HFP (mL)
PCL	-	-	1.3	-	5	5
PCL-PANI	68	87	1.3	-	5	5
PGS	-	-	-	8.0	-	10

Table S2: Primer sequences used for SYBR® Green chemistry based qPCR ((*) means sequences used from Paşca and colleagues[43], (+) means sequences used from Silva and colleagues[58]).

Genes	FORWARD PRIMER SEQUENCE (5' – 3')	REVERSE PRIMER SEQUENCE (5' – 3')
GAPDH(+)	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG
OCT4	GGTCCTTGTGCAGTACTCAGG	CTGGCATAGACGTGAAGAAGC
SOX2	ACCTACAGCATGTCCTACTCG	CTGGGACATGTGAAGTCTGCT
NESTIN	GGACAAGAGAACCTGGAAACAC	TTTCCTTGTCTACCTCCTCTGG
PAX6 (*)	ATGTGTGAGTAAAATTCTGGGCA	GCTTACAACTTCTGGAGTCGCTA
TBR2 (*)	CGCCACCAAACTGAGATGAT	CACATTGTAGTGGGCAGTGG
TBR1	GATCCGAGCTTGTCTTGCAC	AAACTGTGACGAAGCTCAGAGAC
DCX	CCTTGGCTAGCAGCAACAGT	GACAACCCCGGTCTCCAGTT
TUBB3	AGCGGATCAGCGTCTACTAC	CAGGCAGTCGCAGTTTTCAC
NEF-L	TACTCGACCTCCTACAAGCGG	CAGACTGGGCATCAACGATCC
NEF-M	CGAAAGCCACTCAGACCAGAAT	CGGTACTCGGCGATCTCTTCC
NEF-H	CCCCTGCAGACAAATTCCCTG	GGGCTCTTTGACTTTCACCT
MAP2	CAGGGAAGAGTGGTACCTCAAC	CAGGAGATTTTGGAGGAGTACG
Tau	GATGGAAGATCACGCTGGGAC	CAGCAGCTTCGTCTTCCAGG
NCAM	GGATGGCAGTGAGTCAGAGG	CCGGATCTGCAGGTAGTTGT
NEUN	CTGAGATTTATGGAGGCTACGC	CATGGTTCCAATGCTGTAGGTC
SYN	GCCAACAAGACCGAGAGTGAC	GAAGATGTAGGTGGCCAGAGC
VGLUT1	CACTCTAAACATGCTGATCCCC	AACCACAAAAGGCTGTCGTC
VGLUT2	TGGTACTTGCAGTGGGATTCAG	GCAGCGATCAGGAAGACATACT
GAD67	CAAGTTCTGGCTGATGTGGA	CCCTGAGGCTTTGTGGAATA
VGAT	CACGACAAGCCCAAAATCACG	CTCGCCGTCTTCATTCTCCTC
TH	GGAAGGCCGTGCTAAACCT	GTGGATTTTGGCTTCAAACG
DRD2	ATCCTGAACTTGTGTGCCATCA	CGTTCTGGTCTGCGTTATTGAG
CACNA1C	GTACAAAGACGGGGAGGTTGAC	GTAGTTGTAGATGGGGCCCTTG
SCN1α	TTGTGACGCTTAGCCTGGTAG	ACGATGATGGCCAAGACGAG

Table S3: Compilation of the melting/crystallization temperatures obtained in the thermograms for PCL-PANI, PGS raw material and PGS/PCL-PANI fibers (5 ºC min^{-1}, -30 to 100/400 ºC).

		Heating										Cooling / Degradation									
Sample	Cycle #	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})	Peak (º C)	ΔHm (J g^{-1})
PCL-PANI	Cycle 1	-8.1	-0.961	-	-	-	-	35.1	-0.371	60.5	-89.820	36.9	68.81	-	-	-	-	-	-	-	-
	Cycle 2	-	-	-	-	-	-	-	-	58.6	-60.920	36.9	69.79	-	-	-	-	-	-	-	-
	Cycle 3	-	-	-	-	-	-	-	-	58.4	-67.610	155.3	Exothermic	-	-	350.9	Exothermic	378.2	Exothermic	-	-
PGS raw material	Cycle 1	-13.5	-0.995	4.1	-29.38	17.7	-6.104	-	-	-	-	-	-	-	-	-13.4	44.72	-	-	-	-
	Cycle 2	-10.6	-1.202	7.0	-24.38	20.7	-6.354	-	-	-	-	-	-	-	-	-13.4	46.86	-	-	-	-
	Cycle 3	-11.0	-1.735	7.0	-25.45	20.6	-5.932	-	-	-	-	153.3	Exothermic	-	-	-	-	382.8	Exothermic	405.9	Exothermic
PGS/PCL-PANI	Cycle 1	-	-	3.8	-14.25	18.5	-0.812	37.2	-3.630	57.1	-18.390	28.0	18.98	-5.3	24.23	-	-	-	-	-	-
	Cycle 2	-	-	7.7	-14.26	18.7	-2.124	31.6	-0.310	55.4	-22.160	27.8	16.93	-6.3	22.57	-	-	-	-	-	-
	Cycle 3	-	-	7.8	-13.85	19.1	-1.907	31.7	-0.312	55.2	-23.790	157.1	Exothermic	263.2	exothermic	-	-	385.6	Exothermic	409.5	Exothermic

Table S4: Fiber diameter changes across samples from the lipase stability assay (n = 100 measurements; (*) means $p<0.05$ compared to day 0; (+) means $p<0.05$ compared to 1h; (&) means $p<0.05$ compared to 4h; ($) means $p<0.05$ compared to 8h; (§) means $p<0.05$ compared to 12h).

Timepoints	PCL	Monoaxial	Coaxial
Day 0	397 ± 235	296 ± 93	951 ± 465
1h	1035 ± 315 (*)	581 ± 281 (*)	1489 ± 696 (*)
4h	688 ± 326 (*) (+)	587 ± 254 (*)	1135 ± 378 (+)
8h	1335 ± 390 (*) (+) (&)	571 ± 229 (*)	1343 ± 591 (*)
12h	1153 ± 331 (*) (&) ($)	503 ± 142 (*)	1728 ± 870 (*) (&) ($)
24h	-	994 ± 491 (*) (+) (&) ($) (§)	1486 ± 668 (*) (&)
48h	-	1045 ± 346 (*) (+) (&) ($) (§)	1544 ± 698 (*) (&)
168h	-	1078 ± 427 (*) (+) (&) ($) (§)	1704 ± 811 (*) (&) ($)

Table S5: Fiber diameter changes across samples from the PBS stability assay (n = 100 measurements; (*) means $p<0.05$ compared to day 0; (+) means $p<0.05$ compared to day 4; (&) means $p<0.05$ compared to day 7; ($) means $p<0.05$ compared to day 21).

Timepoints	PCL	Monoaxial	Coaxial
Day 0	397 ± 235	296 ± 93	951 ± 465
Day 4	891 ± 344 (*)	671 ± 319 (*)	2400 ± 1733 (*)
Day 7	794 ± 310 (*)	641 ± 273 (*)	1546 ± 941 (*) (+)
Day 14	783 ± 319 (*)	668 ± 292 (*)	2364 ± 1617 (*) (&)
Day 21	857 ± 359 (*)	755 ± 266 (*) (&)	1854 ± 1190 (*)
Day 28	879 ± 266 (*)	747 ± 418 (*)	2595 ± 1887 (*) (&) ($)

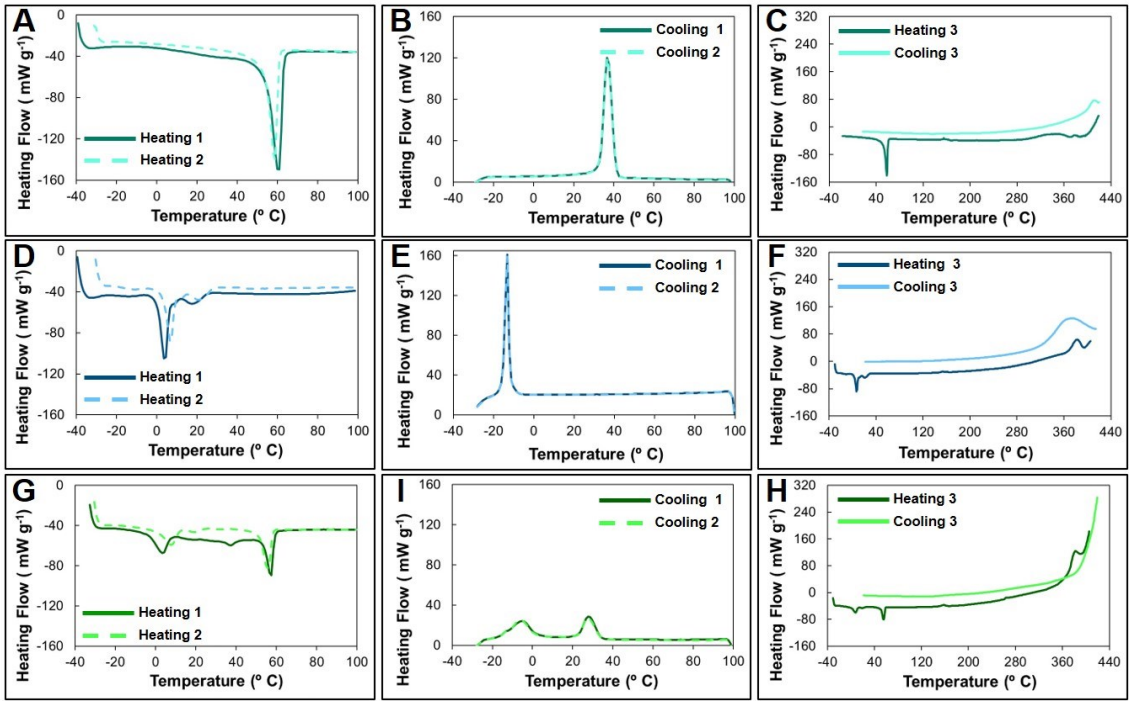

Figure S1: Thermograms for PCL-PANI monoaxial fibers (**A,B,C**), PGS raw material (**D,E,F**), and PGS/PCL-PANI coaxial fibers (**G,H,I**). Sub-figures **A**, **D** and **G** correspond to the heating cycles, **B**, **E** and **H** to the cooling cycles and **C**, **F** and **I** to the last heating and cooling cycles where degradation occurs.

Figure S2: Contact angle images for (**A**) PCL, (**B**) PCL-PANI and (**C**) PGS/PCL-PANI electrospun fibers.

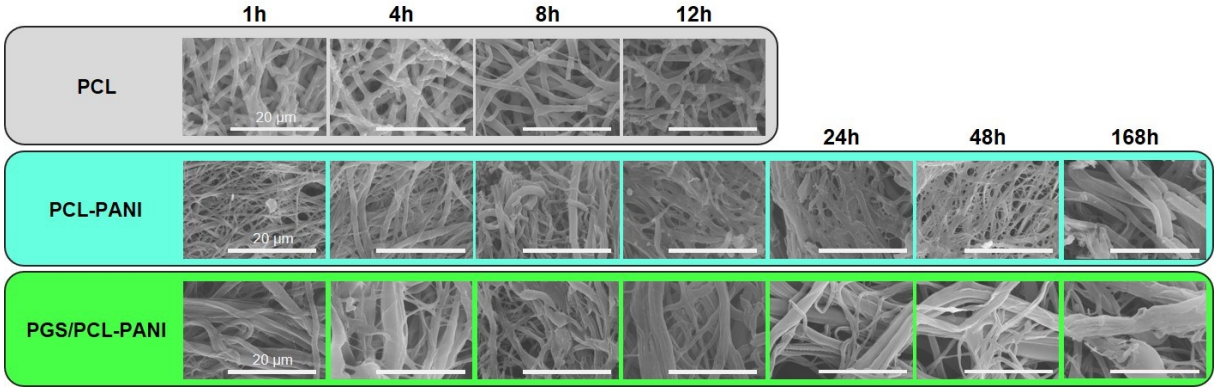

Figure S3: SEM images of fibers obtained at different timepoints of the biodegradability assay performed with lipase (0.5 U mL^{-1}) from *Aspergillus oryzae* (37 ºC and 5 % CO_2).

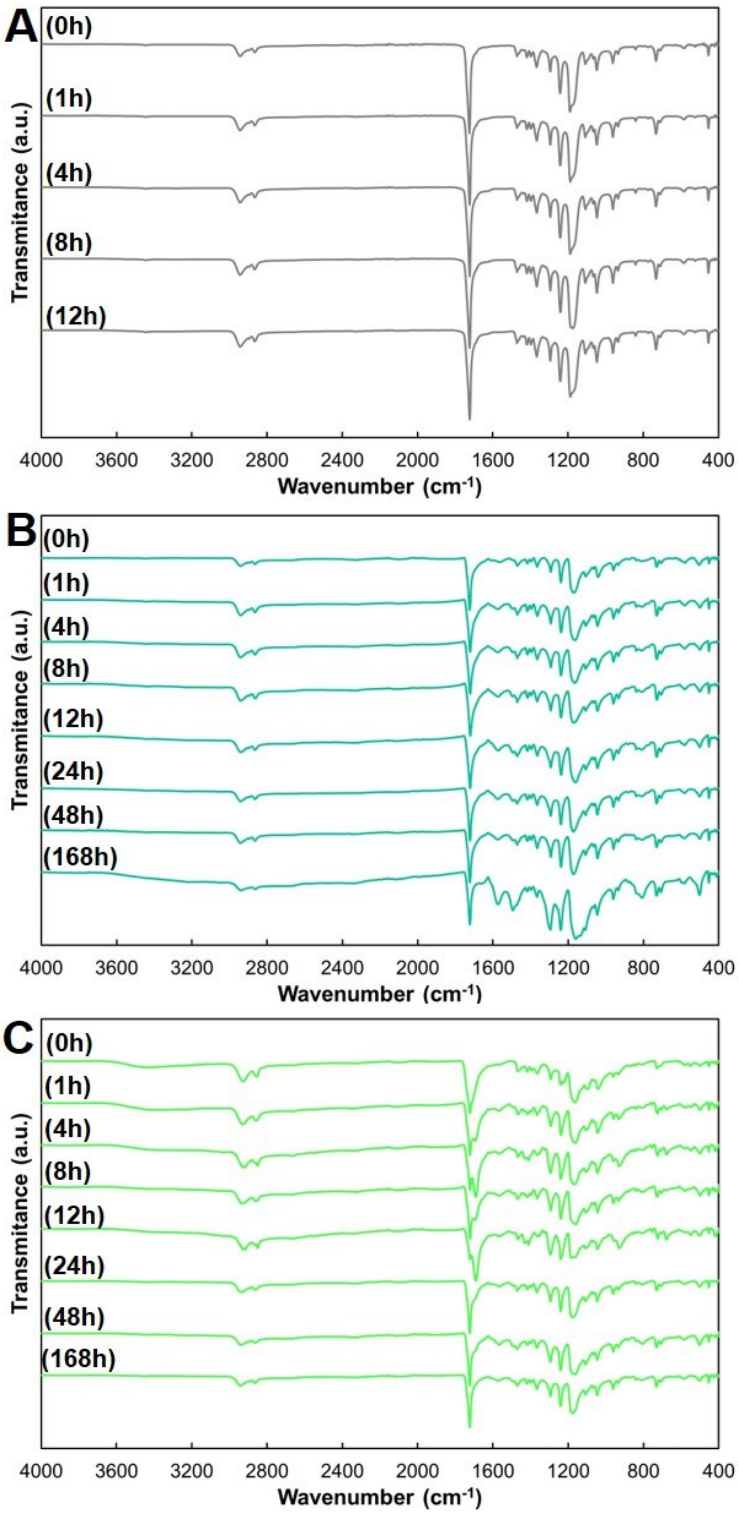

Figure S4: FTIR spectra of the samples obtained during the stability assay with Lipase (0.5 U mL-1) from Aspergillus Oryzae (37 ºC and 5 % CO2). (**A**) PCL, (**B**) PCL-PANI and (**C**) PGS/PCL-PANI (top to bottom) pristine sample (0h) and after incubation for 1 h, 4 h, 8 h, 12 h, 24 h, 48 h and 168 h.

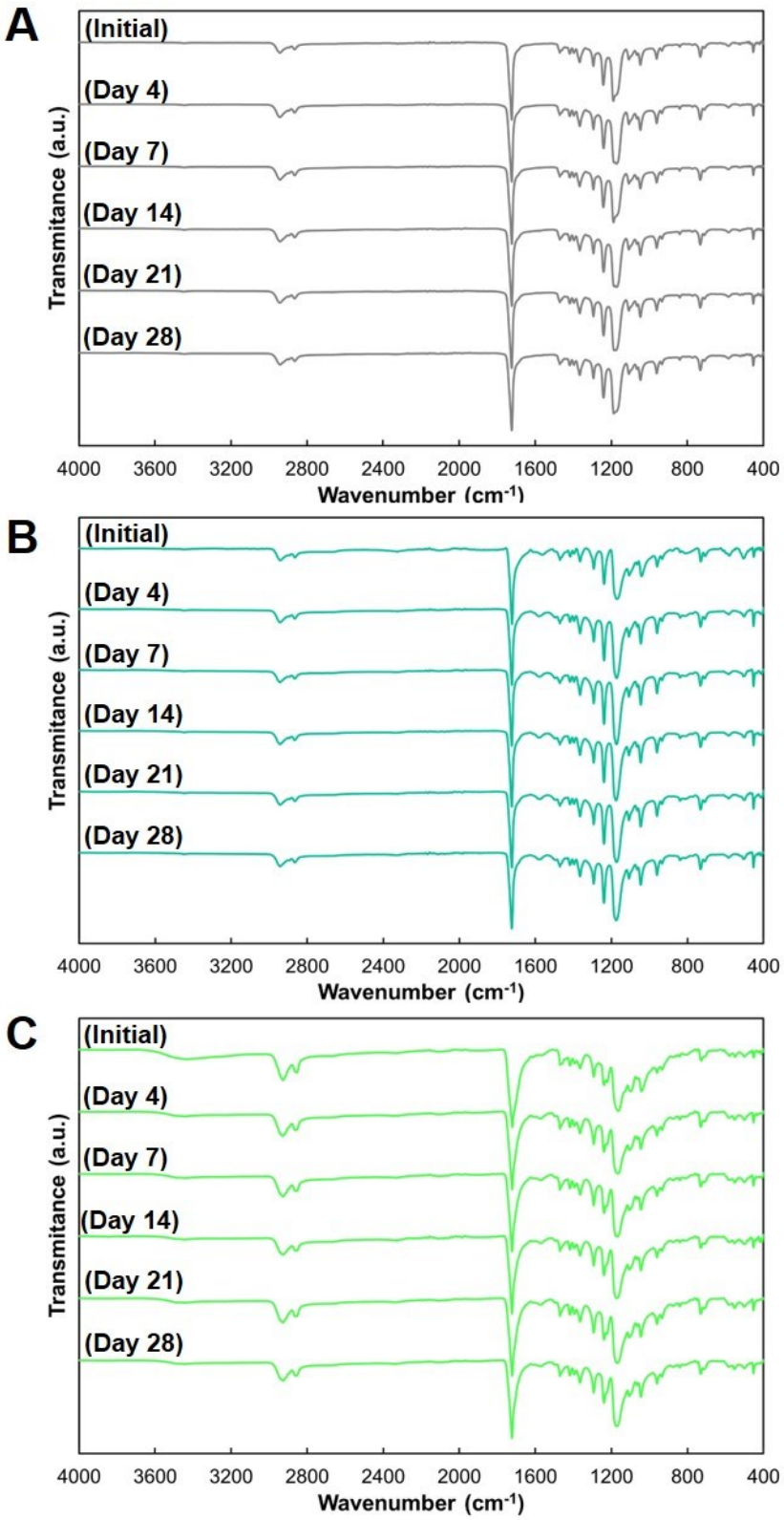

Figure S5: FTIR spectra of (**A**1) PCL, (**A2**) PCL-PANI and (**A3**) PGS/PCL-PANI fibers, including (top to bottom) the initial samples and after incubation periods of 4, 7, 14, 21 and 28 days.

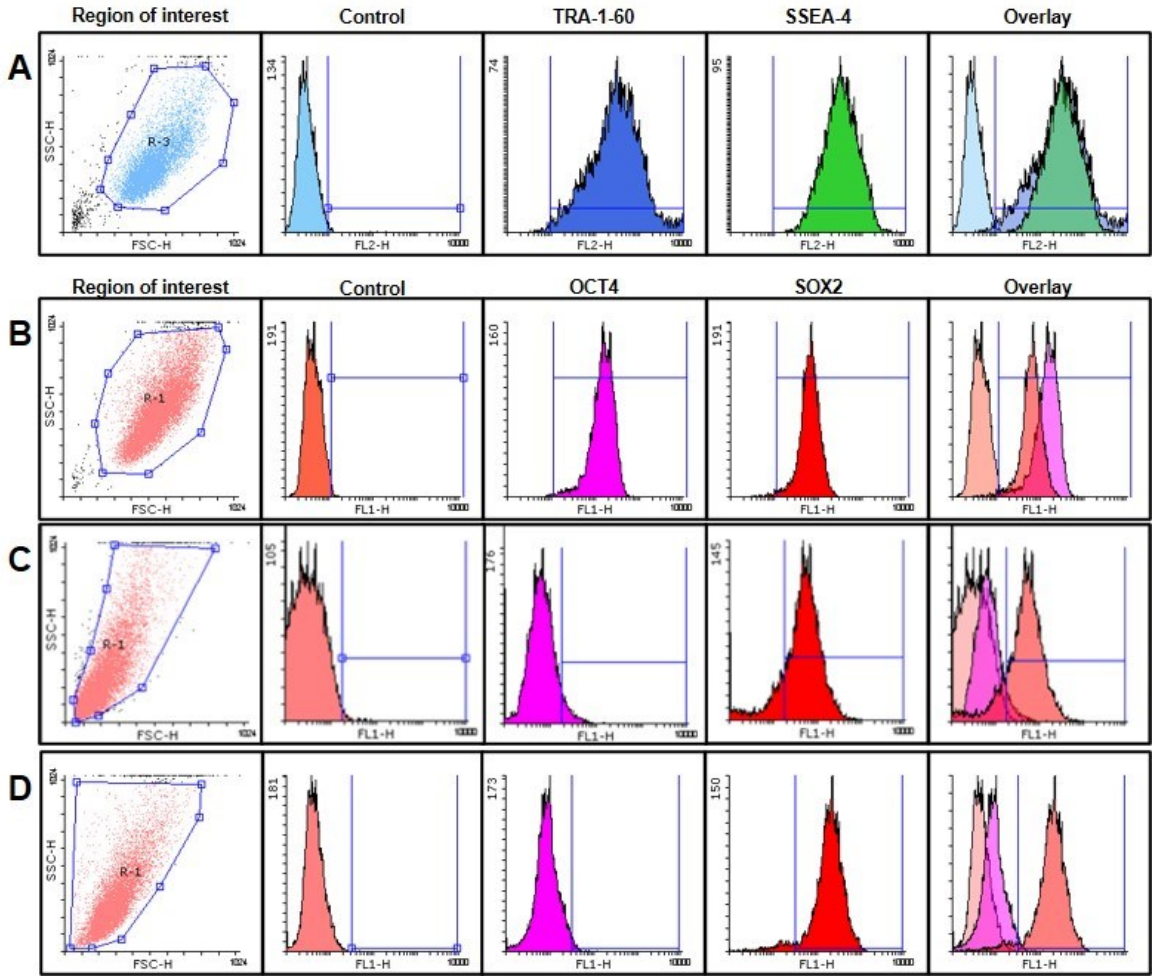

Figure S6: Initial characterization of iPSCs differentiating in iNPCs for 17 days on adherent plates. (**A**) FC profile for TRA-1-60 and SSEA-4 at day 0. (**B**-**D**) FC profile for OCT4 and SOX2 of iPSCs at day 0 (**B**), and differentiation days 12 (**C**) and 17 (**D**).

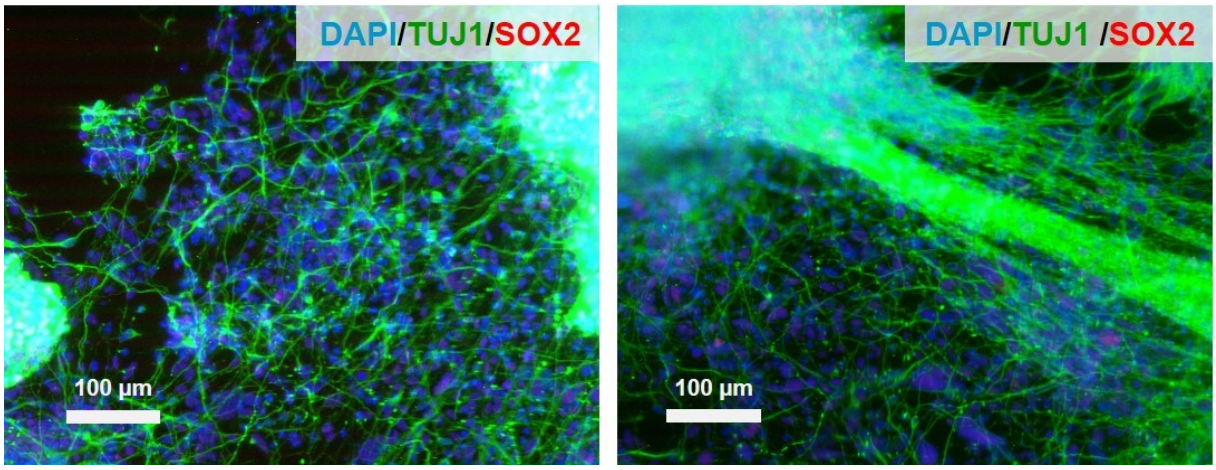

Figure S7: Immunofluorescence images of iNPCs after 30 days of differentiation on the culture tissue plate. Cells were stained for the neural marker Tuj1 and the neural stem cell marker Sox2, and were counter-stained with DAPI.

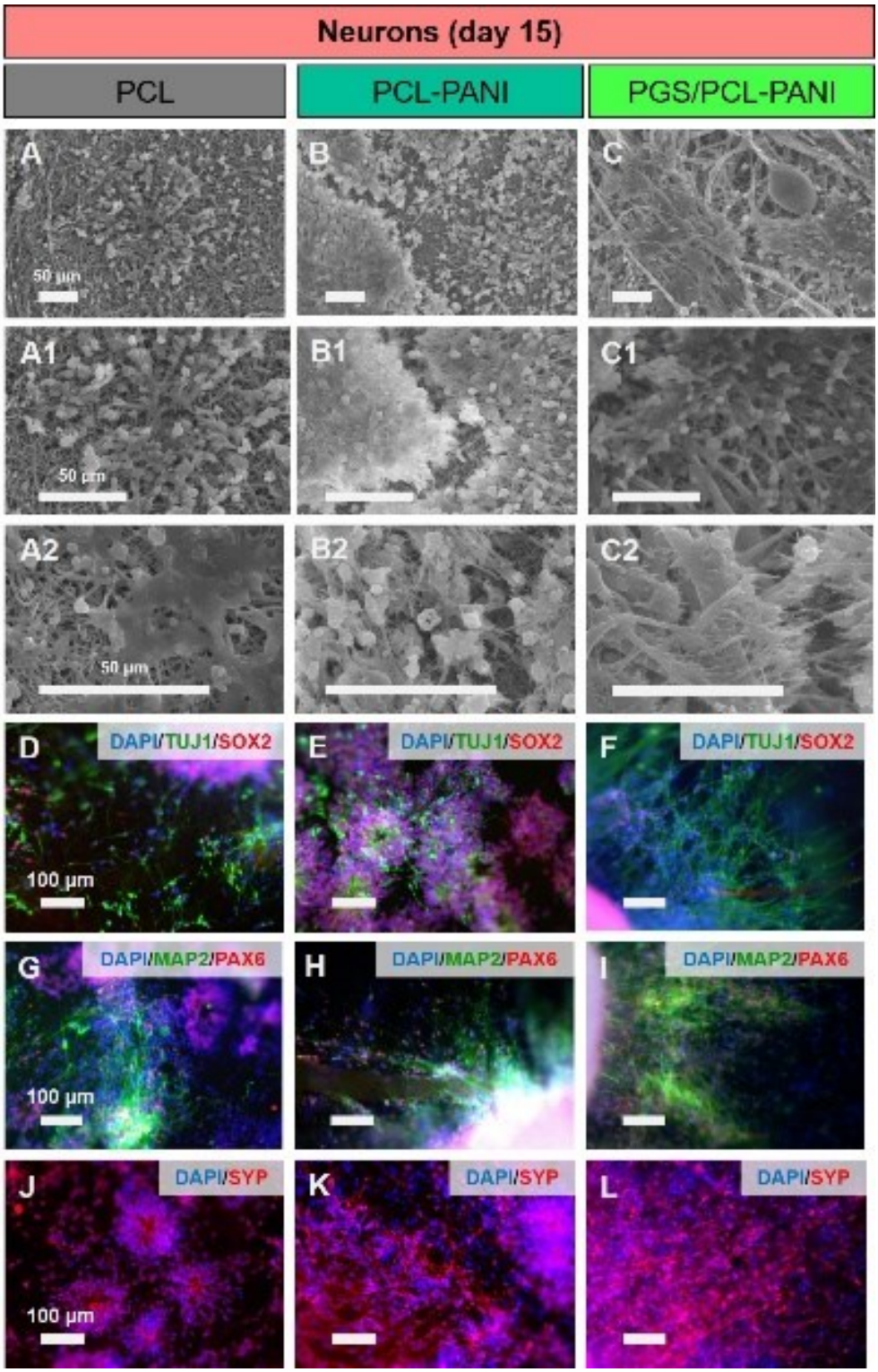

Figure S8: Morphological analysis and marker expression of iNPCs differentiated for 15 days on PCL, PCL-PANI and PGS/PCL-PANI fibers. SEM images, at various magnifications, (**A-C**). IF images for neural marker expression: Tuj1 (**D-F**), Map2 and Pax6 (**G-I**), and Syn (**J-L**), with DAPI as the counter-stain.

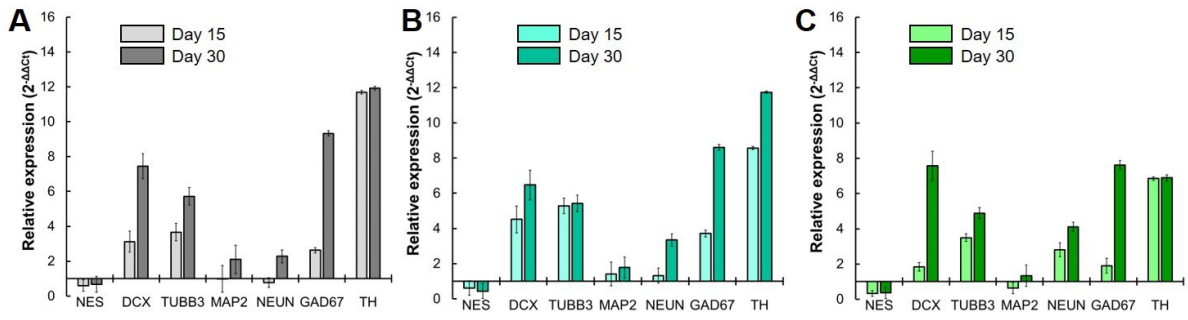

Figure S9: Overall neural markers profile of neurons differentiated for 15 and 30 days on PCL (**A**), PCL-PANI (**B**) and PGS/PCL-PANI (**C**) fibers by qPCR analysis (ΔΔCt method and relative to cell on day 17): NES (neural progenitor cells), DCX and TUBB3 (neurons – early markers), MAP2, NEUN and NCAM (neurons – mature markers), GAD67 (GABAergic neurons), TH and DRD2 (dopaminergic neurons). (mean ± sem, n = 3).

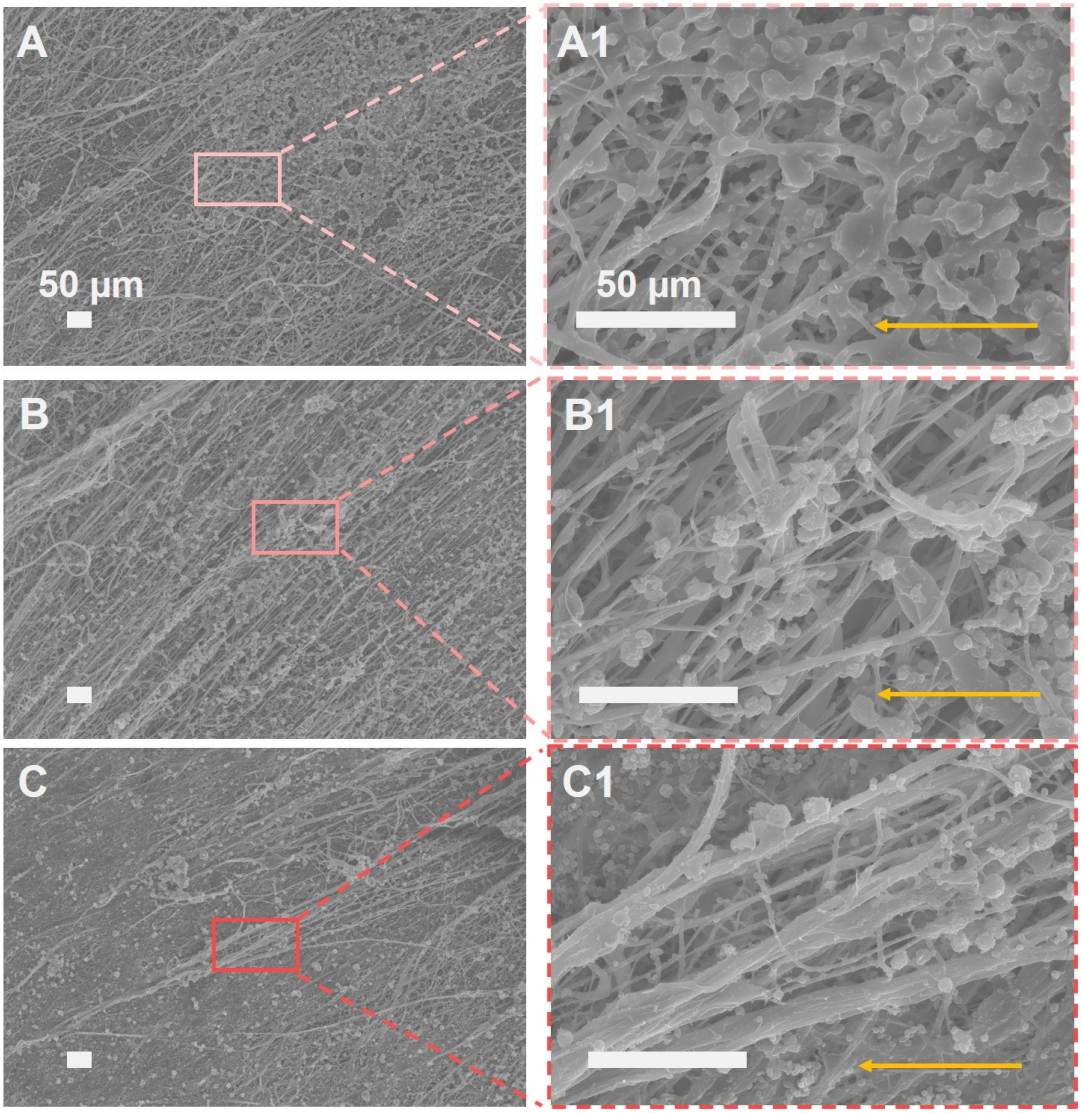

Figure S10: SEM images (**A**, **B**, **C**), and respective close-ups (**A1**, **B1**, **C1**), of neurons differentiated for 30 days on PGS/PCL-PANI fibers under electrical stimulation.